

ADVANCED
NUTRITION
AND HUMAN
METABOLISM

FIFTH EDITION

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Dietary Reference Intakes (DRI)

The Dietary Reference Intakes (DRI) include two sets of values that serve as goals for nutrient intake—Recommended Dietary Allowances (RDA) and Adequate Intakes (AI). The RDA reflect the average daily amount of a nutrient considered adequate to meet the needs of most healthy people. If there is insufficient evidence to determine an RDA, an AI is set. AI are more tentative than RDA, but both may be used as goals for nutrient intakes.

In addition to the values that serve as goals for nutrient intakes, the DRI include a set of values called Tolerable Upper Intake Levels (UL). The UL represent the maximum amount of a nutrient that appears safe for most healthy people to consume on a regular basis.

Estimated Energy Requirements (EER), Recommended Dietary Allowances (RDA), and Adequate Intakes (AI) for Water, Energy, and the Energy Nutrients

Age (yr)	Reference BMI (kg/m ²)	Reference height, cm (in)	Reference weight, kg (lb)	Water ^a , AI (L/day)	Energy EER ^b , (kcal/day)	Carbohydrate RDA (g/day)	Total fiber AI (g/day)	Total fat AI (g/day)	Linoleic acid AI (g/day)	Linolenic acid AI (g/day)	Protein RDA (g/day) ^c	Protein RDA (g/kg/day)
Males												
0–0.5	—	62 (24)	6 (13)	0.7 ^e	570	60	—	31	4.4	0.5	9.1	1.52
0.5–1	—	71 (28)	9 (20)	0.8 ^f	743	95	—	30	4.6	0.5	11	1.2
1–3 ^g	—	86 (34)	12 (27)	1.3	1046	130	19	—	7	0.7	13	1.05
4–8 ^g	15.3	115 (45)	20 (44)	1.7	1742	130	25	—	10	0.9	19	0.95
9–13	17.2	144 (57)	36 (79)	2.4	2279	130	31	—	12	1.2	34	0.95
14–18	20.5	174 (68)	61 (134)	3.3	3152 ^h	130	38	—	16	1.6	52	0.85
19–30	22.5	177 (70)	70 (154)	3.7	3067 ^h	130	38	—	17	1.6	56	0.8
31–50				3.7	3067 ^h	130	38	—	17	1.6	56	0.8
>50				3.7	3067 ^h	130	30	—	14	1.6	56	0.8
Females												
0–0.5	—	62 (24)	6 (13)	0.7 ^e	520	60	—	31	4.4	0.5	9.1	1.52
0.5–1	—	71 (28)	9 (20)	0.8 ^f	676	95	—	30	4.6	0.5	11	1.2
1–3 ^g	—	86 (34)	12 (27)	1.3	992	130	19	—	7	0.7	13	1.05
4–8 ^g	15.3	115 (45)	20 (44)	1.7	1642	130	25	—	10	0.9	19	0.95
9–13	17.4	144 (57)	37 (81)	2.1	2071	130	26	—	10	1.0	34	0.95
14–18	20.4	163 (64)	54 (119)	2.3	2368	130	26	—	11	1.1	46	0.85
19–30	21.5	163 (64)	57 (126)	2.7	2403 ⁱ	130	25	—	12	1.1	46	0.8
>50				2.7	2403 ⁱ	130	21	—	11	1.1	46	0.8
Pregnancy												
1st trimester				3.0	+0	175	28	—	13	1.4	+25	1.1
2nd trimester				3.0	+340	175	28	—	13	1.4	+25	1.1
3rd trimester				3.0	+452	175	28	—	13	1.4	+25	1.1
Lactation												
1st 6 months				3.8	+330	210	29	—	13	1.3	+25	1.3
2nd 6 months				3.8	+400	210	29	—	13	1.3	+25	1.3

NOTE: For all nutrients, values for infants are AI. Dashes indicate that values have not been determined.

^aThe water AI includes drinking water, water in beverages, and water in foods; in general, drinking water and other beverages contribute about 70 to 80 percent, and foods, the remainder. Conversion factors: 1 L = 33.8 fluid oz; 1 L = 1.06 qt; 1 cup = 8 fluid oz.

^bThe Estimated Energy Requirement (EER) represents the average dietary energy intake that will maintain energy balance in a healthy person of a given gender, age, weight, height, and physical activity level. The values listed are based on an “active” person at the reference height and weight and at the midpoint ages for each group until age 19. Chapter 8 provides equations and tables to determine estimated energy requirements.

^cThe linolenic acid referred to in this table and text is the omega-3 fatty acid known as alpha-linolenic acid.

^dThe values listed are based on reference body weights.

^eAssumed to be from human milk.

^fAssumed to be from human milk and complementary foods and beverages. This includes approximately 0.6 L (~3 cups) as total fluid including formula, juices, and drinking water.

^gFor energy, the age groups for young children are 1–2 years and 3–8 years.

^hFor males, subtract 10 kcalories per day for each year of age above 19.

ⁱFor females, subtract 7 kcalories per day for each year of age above 19.

SOURCE: Adapted from the *Dietary Reference Intakes* series, National Academies Press. Copyright 1997, 1998, 2000, 2001, 2002, 2004, 2005 by the National Academies of Sciences.

Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for Vitamins

Age (yr)	Thiamin RDA (mg/day)	Riboflavin RDA (mg/day)	Niacin RDA (mg/day)	Biotin AI (μg/day) ^a	Pantothenic acid AI (mg/day)	Vitamin B ₆ RDA (mg/day)	Folate RDA (μg/day)	Vitamin B ₁₂ RDA (μg/day) ^b	Choline AI (mg/day)	Vitamin C RDA (mg/day)	Vitamin A RDA (μg/day) ^c	Vitamin D AI (μg/day) ^d	Vitamin E RDA (mg/day) ^e	Vitamin F RDA (mg/day)	Vitamin K AI (μg/day)
Infants															
0–0.5	0.2	0.3	2	5	1.7	0.1	65	0.4	125	40	400	5	4	2.0	
0.5–1	0.3	0.4	4	6	1.8	0.3	80	0.5	150	50	500	5	5	2.5	
Children															
1–3	0.5	0.5	6	8	2	0.5	150	0.9	200	15	300	5	6	30	
4–8	0.6	0.6	8	12	3	0.6	200	1.2	250	25	400	5	7	55	
Males															
9–13	0.9	0.9	12	20	4	1.0	300	1.8	375	45	600	5	11	60	
14–18	1.2	1.3	16	25	5	1.3	400	2.4	550	75	900	5	15	75	
19–30	1.2	1.3	16	30	5	1.3	400	2.4	550	90	900	5	15	120	
31–50	1.2	1.3	16	30	5	1.3	400	2.4	550	90	900	5	15	120	
51–70	1.2	1.3	16	30	5	1.7	400	2.4	550	90	900	10	15	120	
>70	1.2	1.3	16	30	5	1.7	400	2.4	550	90	900	15	15	120	
Females															
9–13	0.9	0.9	12	20	4	1.0	300	1.8	375	45	600	5	11	60	
14–18	1.0	1.0	14	25	5	1.2	400	2.4	400	65	700	5	15	75	
19–30	1.1	1.1	14	30	5	1.3	400	2.4	425	75	700	5	15	90	
31–50	1.1	1.1	14	30	5	1.3	400	2.4	425	75	700	5	15	90	
51–70	1.1	1.1	14	30	5	1.5	400	2.4	425	75	700	10	15	90	
>70	1.1	1.1	14	30	5	1.5	400	2.4	425	75	700	15	15	90	
Pregnancy															
≤18	1.4	1.4	18	30	6	1.9	600	2.6	450	80	750	5	15	75	
19–30	1.4	1.4	18	30	6	1.9	600	2.6	450	85	770	5	15	90	
31–50	1.4	1.4	18	30	6	1.9	600	2.6	450	85	770	5	15	90	
Lactation															
≤18	1.4	1.6	17	35	7	2.0	500	2.8	550	115	1200	5	19	75	
19–30	1.4	1.6	17	35	7	2.0	500	2.8	550	120	1300	5	19	90	
31–50	1.4	1.6	17	35	7	2.0	500	2.8	550	120	1300	5	19	90	

NOTE: For all nutrients, values for infants are AI.

^aNiacin recommendations are expressed as niacin equivalents (NE), except for recommendations for infants younger than 6 months, which are expressed as preformed niacin.

^bFolate recommendations are expressed as dietary folate equivalents (DFE).

^cVitamin A recommendations are expressed as retinol activity equivalents (RAE).

^dVitamin D recommendations are expressed as cholecalciferol and assume an absence of adequate exposure to sunlight.

^eVitamin E recommendations are expressed as α-tocopherol.

Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for Minerals

Age (yr)	Sodium AI (mg/day)	Chloride AI (mg/day)	Potassium AI (mg/day)	Calcium AI (mg/day)	Phosphorus RDA (mg/day)	Magnesium RDA (mg/day)	Iron RDA (mg/day)	Zinc RDA (mg/day)	Iodine RDA (μg/day)	Selenium RDA (μg/day)	Copper RDA (μg/day)	Manganese AI (mg/day)	Fluoride AI (mg/day)	Chromium AI (μg/day)	Molybdenum RDA (μg/day)
Infants															
0–0.5	120	180	400	210	100	30	0.27	2	110	15	200	0.003	0.01	0.2	2
0.5–1	370	570	700	270	275	75	11	3	130	20	220	0.6	0.5	5.5	3
Children															
1–3	1000	1500	3000	500	460	80	7	3	90	20	340	1.2	0.7	11	17
4–8	1200	1900	3800	800	500	130	10	5	90	30	440	1.5	1.0	15	22
Males															
9–13	1500	2300	4500	1300	1250	240	8	8	120	40	700	1.9	2	25	34
14–18	1500	2300	4700	1300	1250	410	11	11	150	55	890	2.2	3	35	43
19–30	1500	2300	4700	1000	700	400	8	11	150	55	900	2.3	4	35	45
31–50	1500	2300	4700	1000	700	420	8	11	150	55	900	2.3	4	35	45
51–70	1300	2000	4700	1200	700	420	8	11	150	55	900	2.3	4	30	45
>70	1200	1800	4700	1200	700	420	8	11	150	55	900	2.3	4	30	45
Females															
9–13	1500	2300	4500	1300	1250	240	8	8	120	40	700	1.6	2	21	34
14–18	1500	2300	4700	1300	1250	360	15	9	150	55	890	1.6	3	24	43
19–30	1500	2300	4700	1000	700	310	18	8	150	55	900	1.8	3	25	45
31–50	1500	2300	4700	1000	700	320	18	8	150	55	900	1.8	3	25	45
51–70	1300	2000	4700	1200	700	320	8	8	150	55	900	1.8	3	20	45
>70	1200	1800	4700	1200	700	320	8	8	150	55	900	1.8	3	20	45
Pregnancy															
≤18	1500	2300	4700	1300	1250	400	27	12	220	60	1000	2.0	3	29	50
19–30	1500	2300	4700	1000	700	350	27	11	220	60	1000	2.0	3	30	50
31–50	1500	2300	4700	1000	700	360	27	11	220	60	1000	2.0	3	30	50
Lactation															
≤18	1500	2300	5100	1300	1250	360	10	13	290	70	1300	2.6	3	44	50
19–30	1500	2300	5100	1000	700	310	9	12	290	70	1300	2.6	3	45	50
31–50	1500	2300	5100	1000	700	320	9	12	290	70	1300	2.6	3	45	50

Tolerable Upper Intake Levels (UL) for Vitamins

Age (yr)	Niacin (mg/day) ^a	Vitamin B ₆ (mg/day)	Folate (µg/day) ^a	Choline (mg/day)	Vitamin C (mg/day)	Vitamin A (µg/day) ^b	Vitamin D (µg/day)	Vitamin E (mg/day) ^c
Infants								
0–0.5	—	—	—	—	600	25	—	—
0.5–1	—	—	—	—	600	25	—	—
Children								
1–3	10	30	300	1000	400	600	50	200
4–8	15	40	400	1000	650	900	50	300
9–13	20	60	600	2000	1200	1700	50	600
Adolescents								
14–18	30	80	800	3000	1800	2800	50	800
Adults								
19–70	35	100	1000	3500	2000	3000	50	1000
>70	35	100	1000	3500	2000	3000	50	1000
Pregnancy								
≤18	30	80	800	3000	1800	2800	50	800
19–50	35	100	1000	3500	2000	3000	50	1000
Lactation								
≤18	30	80	800	3000	1800	2800	50	800
19–50	35	100	1000	3500	2000	3000	50	1000

^aThe UL for niacin and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.

^bThe UL for vitamin A applies to the preformed vitamin only.

^cThe UL for vitamin E applies to any form of supplemental α-tocopherol, fortified foods, or a combination of the two.

Tolerable Upper Intake Levels (UL) for Minerals

Age (yr)	Sodium (mg/day)	Chloride (mg/day)	Calcium (mg/day)	Phosphorus (mg/day)	Magnesium (mg/day) ^d	Iron (mg/day)	Zinc (mg/day)	Iodine (µg/day)	Selenium (µg/day)	Copper (µg/day)	Manganese (mg/day)	Fluoride (mg/day)	Molybdenum (µg/day)	Boron (mg/day)	Nickel (mg/day)	Vanadium (mg/day)
Infants																
0–0.5	— ^e	— ^e	—	—	40	4	—	45	—	—	0.7	—	—	—	—	—
0.5–1	— ^e	— ^e	—	—	40	5	—	60	—	—	0.9	—	—	—	—	—
Children																
1–3	1500	2300	2500	3000	65	40	7	200	90	1000	2	1.3	300	3	0.2	—
4–8	1900	2900	2500	3000	110	40	12	300	150	3000	3	2.2	600	6	0.3	—
9–13	2200	3400	2500	4000	350	40	23	600	280	5000	6	10	1100	11	0.6	—
Adolescents																
14–18	2300	3600	2500	4000	350	45	34	900	400	8000	9	10	1700	17	1.0	—
Adults																
19–70	2300	3600	2500	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8
>70	2300	3600	2500	3000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8
Pregnancy																
≤18	2300	3600	2500	3500	350	45	34	900	400	8000	9	10	1700	17	1.0	—
19–50	2300	3600	2500	3500	350	45	40	1100	400	10,000	11	10	2000	20	1.0	—
Lactation																
≤18	2300	3600	2500	4000	350	45	34	900	400	8000	9	10	1700	17	1.0	—
19–50	2300	3600	2500	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	—

^dThe UL for magnesium applies to synthetic forms obtained from supplements or drugs only.

^eSource of intake should be from human milk (or formula) and food only.

NOTE: An Upper Limit was not established for vitamins and minerals not listed and for those age groups listed with a dash (—) because of a lack of data, not because these nutrients are safe to consume at any level of intake. All nutrients can have adverse effects when intakes are excessive.

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*To my parents for their love, support, and encouragement.
To Michelle and Michael, who keep my life balanced and give me great joy.
To my husband, Daniel, for his ongoing encouragement, support, faith, and love.*

Sareen Gropper

*To my wife, Carol, for her support for my coauthoring this new edition and for
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the changes in my career and our new directions.*

Jack Smith

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Preface

Since the first edition was published in 1990, much has changed in the science of nutrition. But the purpose of the text—to provide thorough coverage of normal metabolism for upper-division nutrition students—remains the same. We continue to strive for a level of detail and scope of material that satisfy the needs of both instructors and students. With each succeeding edition, we have responded to suggestions from instructors, content reviewers, and students that have improved the text by enhancing the clarity of the material and by ensuring accuracy. In addition, we have included the latest and most pertinent nutrition science available to provide future nutrition professionals with the fundamental information vital to their careers and to provide the basis for assimilating new scientific discoveries as they happen.

Just as the body of information on nutrition science has increased, so has the team of authors working on this text. Dr. James Groff and Dr. Sara Hunt coauthored the first edition. In subsequent editions, Dr. Sareen Gropper became a coauthor as Dr. Sara Hunt entered retirement. In the fourth edition, Dr. Jack L. Smith joined the author team. Drs. Gropper and Smith have continued to devote their efforts and time in coauthoring this fifth edition.

NEW TO THIS EDITION

In this edition, we worked to improve the clarity of the figures and the two-color design introduced last edition to visually emphasize important concepts in the chapters. The second color is better used in the figures to highlight reactants, products, and movement through the biochemical pathways. We hope this use of color will improve student learning. In addition, we reorganized some content between chapters to improve organization. This restructuring resulted in one less chapter and should improve readability.

While the chapter text continues to concentrate on normal nutrition and physiological function, we have tried to provide more connections between normal and clinical nutrition and between physiology and pathophysiology. The Perspectives continue to deal with clinical, pathological, and applied aspects germane to the subject of each corresponding chapter.

PRESENTATION

The presentation of the fifth edition is designed to make the book easier for the reader to use. The second color draws attention to important elements in the text, tables, and figures and helps generate reader interest. The Perspectives provide applications of the information in the chapter text and have been well received by reviewers and users.

Because this book focuses on normal human nutrition and physiological function, it is an effective resource for students majoring in either nutrition sciences or dietetics. Intended for a course in advanced nutrition, the text presumes a sound background in the biological sciences. At the same time, however, it provides a review of the basic sciences—particularly biochemistry and physiology, which are important to understanding the material. This text applies biochemistry to nutrient use from consumption through digestion, absorption, distribution, and cellular metabolism, making it a valuable reference for health care workers. Health practitioners may use it as a resource to refresh their memories with regard to metabolic and physiological interrelationships and to obtain a concise update on current concepts related to human nutrition.

We continue to present nutrition as the science that integrates life processes from the molecular to the cellular level and on through the multisystem operation of the whole organism. Our primary goal is to give a comprehensive picture of cell reactions at the tissue, organ, and system levels. Subject matter has been selected for its relevance to meeting this goal.

ORGANIZATION

Each of the 15 chapters begins with a topic outline, followed by a brief introduction to the chapter's subject matter. These features are followed in order by the chapter text, a brief summary that ties together the ideas presented in the chapter, a reference list, and a Perspective with its own reference list.

The text is divided into five sections. Section I (Chapters 1 and 2) focuses on cell structure, gastrointestinal tract

anatomy, and function with respect to digestion and absorption. The information contained in the energy transformation chapter of previous editions has been split between the chapter on the cell (Chapter 1) and the carbohydrates chapter (Chapter 3). This reorganization associates similar information more closely. Most of the body's energy production is associated with glycolysis or the tricarboxylic acid cycle by the way of the electron transport and oxidative phosphorylation.

Section II (Chapters 3–8) discusses the metabolism of the macronutrients. This section reviews primary metabolic pathways for carbohydrates, lipids, and proteins, emphasizing those reactions particularly relevant to issues of health. We include a separate chapter on fiber. The discussion of alcohol metabolism has been moved from the carbohydrates chapter (Chapter 3) to the lipids chapter (Chapter 5). Alcohol contributes to the caloric intake of many people. Its chemical structure more closely resembles that of carbohydrates, but its metabolism is more similar to that of lipids. Chapter 7 discusses the interrelationships among the metabolic pathways that are common to the macronutrients. This chapter also includes a discussion of the regulation of the metabolic pathways and a description of the metabolic dynamics of the fed-fast cycle, along with a presentation of exercise and sports nutrition and the effects of physical exertion on the body's metabolic pathways. The chapter on body composition (Chapter 8) has been moved into this section. It emphasizes energy balance and the influence of energy balance on the various body compartments. This chapter also includes a brief discussion of hormonal control of food intake, the prevalence of obesity, and the regulation of body weight. The information on the change of body composition through development has been moved to the protein chapter (Chapter 6).

Section III (Chapters 9–13) concerns those nutrients considered regulatory in nature: the water- and fat-soluble vitamins and the minerals, including the macrominerals, micro-minerals, and ultratrace minerals. These chapters cover nutrient features such as digestion, absorption, transport, function, metabolism, excretion, deficiency, toxicity, and assessment of nutriture, as well as the latest recommended dietary allowances or adequate intakes for each nutrient.

Section IV (Chapter 14) covers the maintenance of the body's homeostatic environment. It includes discussion of body fluids and electrolyte balance, and pH maintenance. The final chapter (Chapter 15), "Experimental Design and Critical Interpretation of Research," has been condensed. It constitutes Section V and is supplementary to the rest of the book. This chapter discusses the types of research and the methodologies by which research can be conducted. It is designed to familiarize students with research organization and implementation, to point out problems and pitfalls inherent in research, and to help students critically evaluate scientific literature.

SUPPLEMENTARY MATERIAL

To enhance teaching and learning from the textbook, a Multimedia Manager CD-ROM is available. This multimedia collection of visual resources provides instructors with the complete collection of figures from the textbook. Instructors may use illustrations to create custom classroom presentations, visually based tests and quizzes, or classroom support materials. In addition, a robust test bank is available both electronically on the Multimedia Manager CD-ROM and in printed form. Students will find study guide resources and online practice tests for each chapter on the book's companion web site.

ACKNOWLEDGMENTS

Although this textbook represents countless hours of work by the authors, it is also the work of many other hardworking individuals. We cannot possibly list everyone who has helped, but we would like to call attention to a few individuals who have played particularly important roles. We thank our undergraduate and graduate nutrition students for their ongoing feedback. We thank the executive editor, Peter Adams; our developmental editor, Anna Lustig; our associate development editor, Elesha Feldman; our art director, John Walker; our marketing manager, Jennifer Somerville; our content project manager for editorial production, Jennifer Risdin; and our permissions editors, Sue Howard and Mardell Glinski Schultz. We extend special thanks to our production team, especially Katy Bastille, and our copy editor, Alison Darrow.

We thank two additional contributors, who also worked with us on the fifth edition of the text: Ruth M. DeBusk, Ph.D., R.D., for writing the Perspective "Nutritional Genomics: The Foundation for Personalized Nutrition," and Rita M. Johnson, Ph.D., R.D., F.A.D.A., for the Perspective, "Genetics and Nutrition: The Possible Effect on Folate Needs and Risk of Chronic Disease."

We are indebted to the efforts of Carole A. Conn (University of New Mexico), who was lead author for the test bank; Kevin Schalinske (Iowa State University), who wrote the study guide for the student Web site and contributed to the test bank; and Mary Jacob (California State University, Long Beach), who contributed to the test bank.

We owe special thanks to the reviewers whose thoughtful comments, criticisms, and suggestions were indispensable in shaping this text.

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1

The Cell: A Microcosm of Life

Components of Typical Cells

- Plasma Membrane
- Cytoplasmic Matrix
- Mitochondrion
- Nucleus
- Endoplasmic Reticulum and Golgi Apparatus
- Lysosomes and Peroxisomes

Cellular Proteins

- Receptors and Intracellular Signaling
- Transport Proteins
- Catalytic Proteins (Enzymes)
- Practical Clinical Application of Cellular Enzymes

Apoptosis

- Programmed Death
- Potential Mechanisms

Biological Energy

- Energy Release and Consumption in Chemical Reactions
- Units of Energy
- The Role of High-Energy Phosphate in Energy Storage
- Coupled Reactions in the Transfer of Energy
- Reduction Potentials

PERSPECTIVE

Nutritional Genomics : The Foundation for Personalized Nutrition

Cells are the very essence of life. Cells may be defined as the basic living, structural, and functional units of the human body. They vary greatly in size, chemical composition, and function, but each one is a remarkable miniaturization of human life. Cells move, grow, ingest food and excrete wastes, react to their environment, and even reproduce. This chapter provides a brief review of the basics of a cell, including cellular components, communication, energy, and transport. An overview of the natural life span of a typical cell is provided because of its importance in nutrition and disease.

Cells of all multicellular organisms are called **eukaryotic cells** (from the Greek *eu*, meaning “true,” and *karyon*, “nucleus”). Eukaryotic cells evolved from simpler, more primitive cells called **prokaryotic cells**. The major distinguishing feature between the two cell types is that eukaryotic cells possess a defined nucleus, whereas prokaryotic cells do not. Also, eukaryotic cells are larger and much more complex structurally and functionally than their ancestors. Because this text addresses human metabolism and nutrition, all descriptions of cellular structure and function in this and subsequent chapters pertain to eukaryotic cells.

Specialization among cells is a necessity for the living, breathing human, but cells in general have certain basic similarities. All human cells have a plasma membrane and a nucleus (or have had a nucleus), and most contain an endoplasmic reticulum, Golgi apparatus, and mitochondria. For convenience of discussion, this book considers a so-called “typical cell” to enable us to identify the various organelles and their functions, which characterize cellular life. Considering the relationship between the normal functioning of a typical cell and the health of the total organism—the human being—brings to mind the old rule: “A chain is only as strong as its weakest link.”

Figure 1.1 shows the fine structure of a typical animal cell. A similar view of a typical animal absorptive cell (such as an intestinal epithelial cell) is included in the discussion of digestion in Chapter 2.

Our discussion begins with the plasma membrane, which forms the outer boundary of the cell, and then moves inward to examine the organelles held within this membrane. This chapter covers the information about molecules in the cell that is needed to understand cell structure and function. The chemical structures of the molecules are described later in the appropriate chapters.

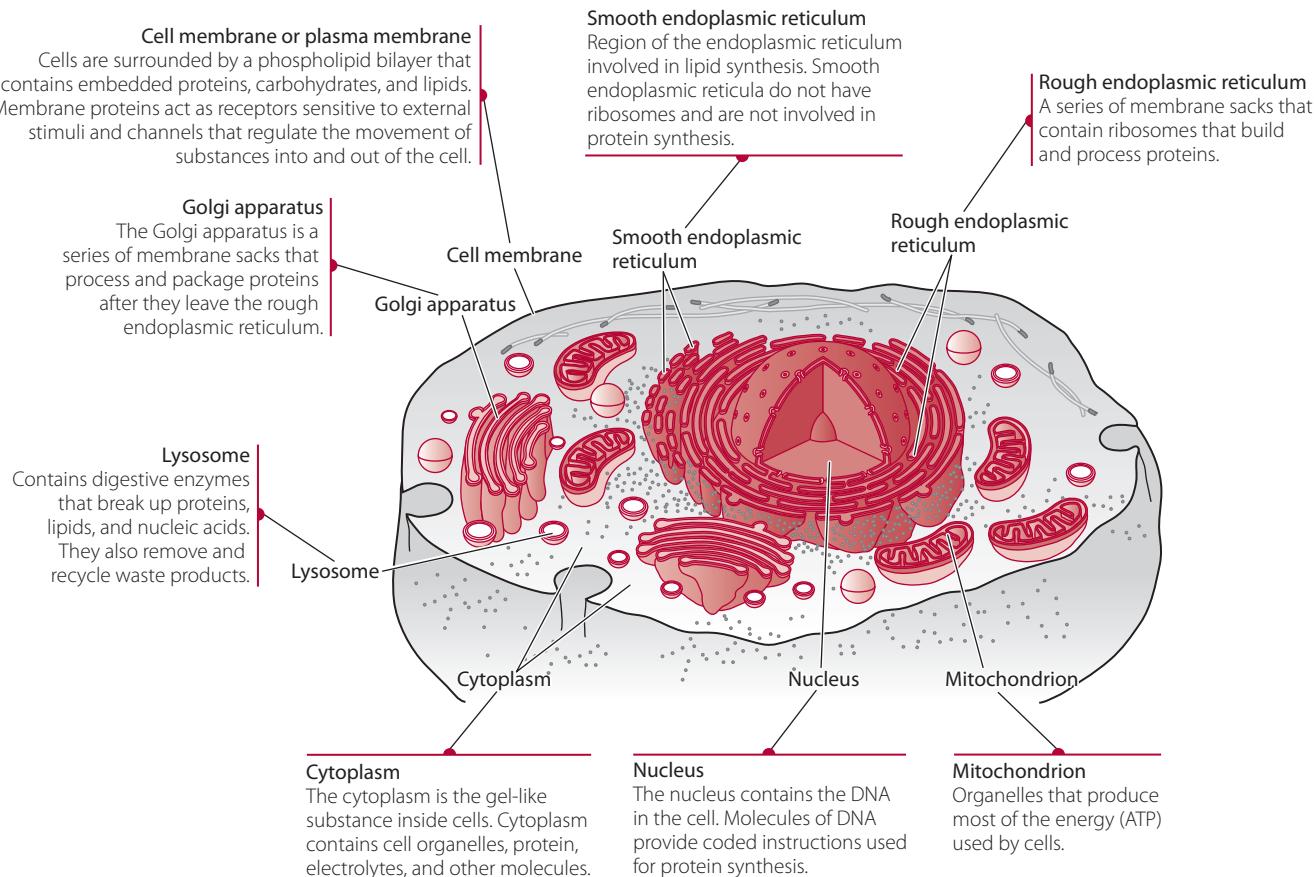


Figure 1.1 Typical animal cell.

Components of Typical Cells

PLASMA MEMBRANE

The **plasma membrane** is the membrane encapsulating the cell. By surrounding the cell, it lets the cell become a unit by itself. The plasma membrane, like other membranes found within the cell, has distinct functions and structural characteristics. Nevertheless, all membranes share some common attributes:

- Membranes are sheetlike structures composed primarily of phospholipids and proteins held together by non-covalent interactions.
- Membrane phospholipids have both a hydrophobic and a hydrophilic moiety. This structural property of phospholipids allows them to spontaneously form bimolecular sheets in water, called lipid bilayers. Figure 1.2 depicts the cellular membrane as it would surround a cell. Figure 1.3 shows a close up of the cell membrane that illustrates several of its functions. Note the phospholipid bilayer and the proteins in the cell membrane, and the

intracellular space inside the cell and extra cellular space outside the cell. The core of the bilayer is hydrophobic, which inhibits many water-soluble compounds from passing into and out of the cell. The integral transport protein shown in this figure is part of a transport system that enables essential water-soluble substances to cross the plasma membrane. The hydrophobic bilayer also helps to retain essential water-soluble substances within the cell.

- Phosphoglycerides and phosphingolipids (phosphate-containing sphingolipids) comprise most of the membrane phospholipids. Chemical structures and properties of the phospholipids in the cellular membrane are described more fully in Chapter 5. Of the phosphoglycerides, phosphatidylcholine and phosphatidylethanolamine are particularly abundant in higher animals. Another important membrane lipid is cholesterol, but its amount varies considerably from membrane to membrane. Cholesterol is present in the hydrophobic portion of the bilayer.
- Membrane proteins give biological membranes their functions: They serve as pumps, gates, receptors, energy transducers, and enzymes. These functions are represented in Figure 1.3. Many of these proteins have either lipid or carbohydrate attachments.

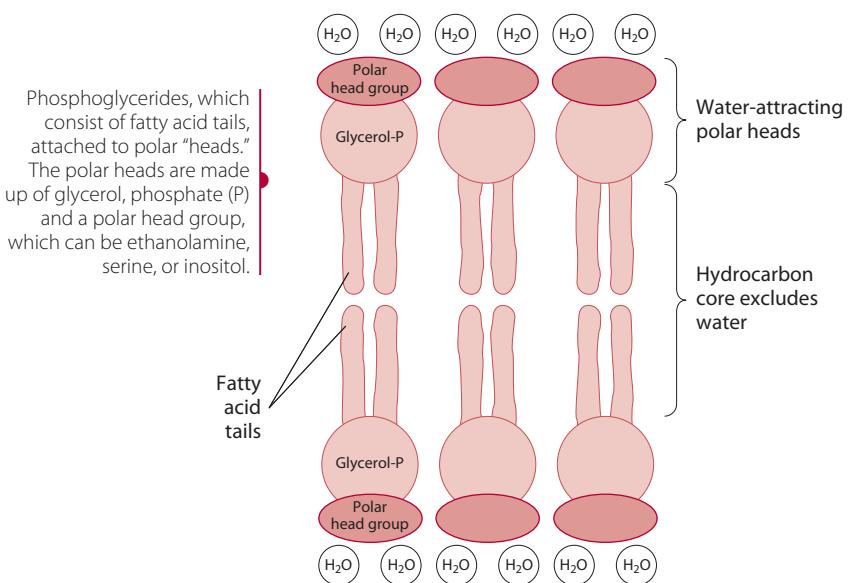


Figure 1.2 Lipid bilayer structure of biological membranes.

- Membranes are asymmetrical. The inside and outside faces of the membrane are different.
- Membranes are not static but are fluid structures. The lipid and protein molecules within them move laterally with ease and rapidity.

Membranes are not structurally distinct from the aqueous compartments of the cell they surround. For example, the **cytoplasm**, which is a gel-like, aqueous, transparent substance that fills the cell, connects the various membranes of the cell. This interconnection creates a structure that makes it possible for a signal generated at one part of

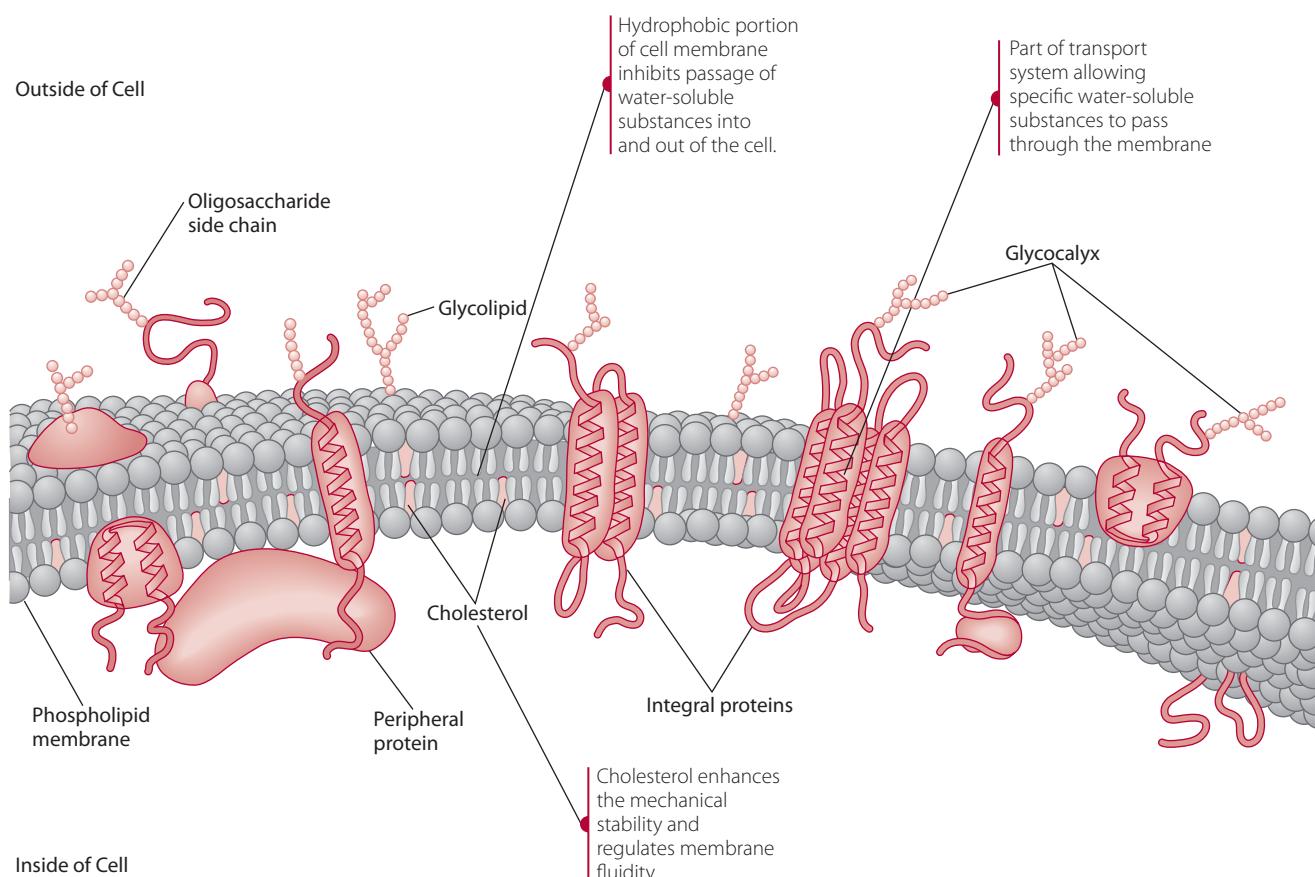


Figure 1.3 Fluid model of cell membrane. Lipids and proteins are mobile. They can move laterally in the membrane.

the cell to be transmitted quickly and efficiently to other regions of the cell.

The plasma membrane protects the cellular components while at the same time allowing them sufficient exposure to their environment for stimulation, nourishment, and removal of wastes. Plasma membranes are chemically distinct from other membranes. Plasma membranes have:

- Greater carbohydrate content, due to the presence of glycolipids and glycoproteins. Some carbohydrate is found in all membranes, but most of the glycolipids and glycoproteins of the cell are associated with the plasma membrane.
- Greater cholesterol content. Cholesterol enhances the mechanical stability of the membrane and regulates its fluidity.

Figure 1.3 illustrates the position of a cholesterol molecule between two phospholipid molecules. The hydrocarbon side chain of the cholesterol molecule associates with the hydrocarbon fatty acid tails of the phospholipids, creating a hydrophobic region. The hydroxyl groups of the cholesterol are positioned close to the polar head groups of the phospholipid molecules, resulting in a more hydrophilic region [1,2]. This layering of polar and nonpolar regions has led to the concept of the lipid bilayer to describe the plasma membrane structure. The cholesterol's rigid planar steroid rings are positioned so as to interact with and stabilize those regions of the hydrocarbon chains closest to the polar head groups. The rest of the hydrocarbon chain remains flexible and fluid. Cholesterol, by regulating fluidity of the membrane, regulates membrane permeability, thereby exercising some control over what may pass into and out of the cell. The fluidity of the membrane also appears to affect the structure and function of the proteins embedded in the lipid membrane.

The carbohydrate moiety of the glycoproteins and the glycolipids in membranes helps maintain the asymmetry of the membrane, because the oligosaccharide side chains are located exclusively on the membrane layer facing away from the cytoplasmic matrix. In plasma membranes, therefore, the sugar residues are all exposed to the outside of the cell, forming what is called the **glycocalyx**, the layer of carbohydrate on the cell's outer surface. On the membranes of the organelles, however, the oligosaccharides are directed inward, into the lumen of the membrane-bound compartment. Figure 1.3 illustrates the glycocalyx and the location of oligosaccharide side chains in the plasma membrane.

Although the exact function of the sugar residues is unknown, they are believed to act as specificity markers for the cell and as "antennae" to pick up signals for transmission of substances in the cell. The membrane glycoproteins are crucial to the life of the cell, very possibly serving as the receptors for hormones, certain nutrients,

and various other substances that influence cellular function. Glycoproteins also may help regulate the intracellular communication necessary for cell growth and tissue formation. Intracellular communication occurs through pathways that convert information from one part of a cell to another in response to external stimuli. Generally, it involves the passage of chemical messengers from organelle to organelle or within the lipid bilayers of membranes. Intracellular communication is examined more closely in the "Receptors and Intracellular Signaling" section of this chapter.

Whereas the lipid bilayer determines the structure of the plasma membrane, proteins are primarily responsible for the many membrane functions. The membrane proteins are interspersed within the lipid bilayer, where they mediate information transfer (as receptors), transport ions and molecules (as channels, carriers, and pumps), and speed up metabolic activities (as enzymes). Figure 1.3 illustrates the integral proteins which are involved in transporting molecules into and out of the cell.

Membrane proteins are classified as either integral or peripheral. The integral proteins are attached to the membrane through hydrophobic interactions and are embedded in the membrane. Peripheral proteins, in contrast, are associated with membranes through ionic interactions and are located on or near the membrane surface (Figure 1.3). Peripheral proteins are believed to be attached to integral membrane proteins either directly or through intermediate proteins [1,2].

Most receptor and carrier proteins are integral proteins, whereas the glycoproteins of the cell recognition complex are peripheral proteins [1]. Functions of membrane proteins, as well as functions of proteins located intracellularly, are described later in this chapter.

CYTOPLASMIC MATRIX

The advent of the electron microscope opened a new frontier in the study of cell structure and cell physiology. This microscope was able to identify the microtrabecular lattice, a fibrous web of connective tissue that supports and controls the movement of cell organelles. Figure 1.4 shows the spatial relationship of the components of the cytoplasm. An intricate network of protein filaments extends throughout the cell and provides support within the cytoplasm. The microtubules are proteins that underlie the plasma membrane, the surface of the endoplasmic reticulum. The lattice appears to support certain extracellular extensions emanating from the cell surface. For example, the **microvilli**, which are extensions of intestinal epithelial cells, are associated with the microtrabeculae. Microvilli are designed to present a large surface area to absorb dietary nutrients. Microtubules, together with a network of filaments that interconnect them, form the **cytoskeleton**. The cytoskeleton is a part of the cellular matrix, most commonly called the cytoplasm.

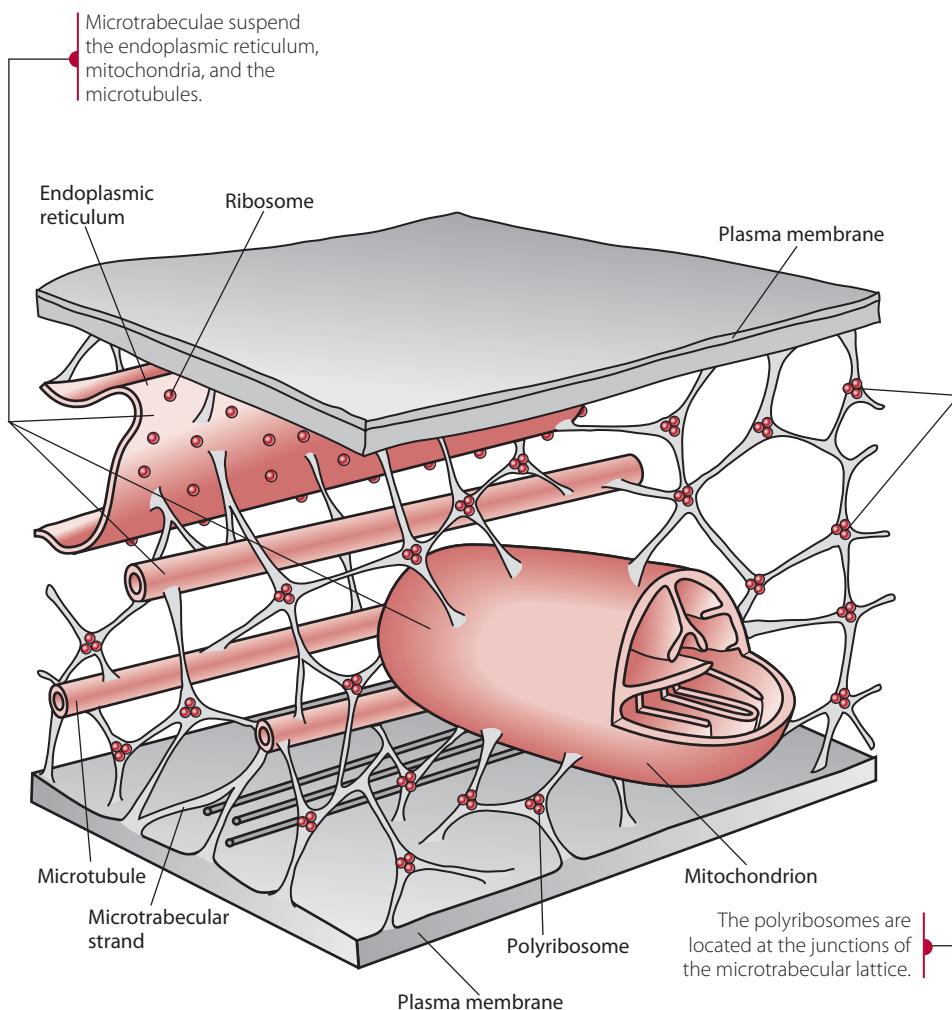


Figure 1.4 The cytoskeleton (microtrabecular lattice) provides a structure for cell organelles, microvilli (as found in intestinal mucosa cells), and large molecules. The cytoplasm is shown at about 300,000 times its actual size and was derived from hundreds of images of cultured cells viewed in a high-voltage electron microscope.

Microfilaments and microtubules are complex polymers of many different proteins, including actin, myosin, and tubulin, the last of which is a protein necessary to form microtubules. These structures provide mechanical support for the cell and also serve as binding surfaces for soluble macromolecules, such as proteins and nucleic acids, that are present in the aqueous portion of the cytoplasmic matrix. The interior of the cell is in continuous motion, and the cytoskeleton provides the machinery for intracellular movement. The nonfilamentous aqueous portion of the cell contains very few macromolecules and that many proteins in the cytoplasmic matrix are bound to the filaments for a large portion of their lives. The fluid portion of the cytoplasmic matrix not associated with the microtubules contains small molecules such as glucose, amino acids, oxygen, and carbon dioxide. This arrangement of the polymeric and fluid portions apparently gives the cytoplasm its gel-like consistency.

Figure 1.5 summarizes the structures of a cell in a three-dimensional model. The spatial arrangement of the cytoskeleton (which also includes the microfilaments)

with the aqueous phase of the cell improves the efficiency of the many enzyme-catalyzed reactions that take place in the cytoplasm. Because the aqueous part of the cell contacts with the cytoskeleton over a very broad surface area, enzymes that are associated with the polymeric lattice are brought into close proximity to their substrate molecules in the aqueous portion, thereby facilitating the reaction [see the “Catalytic Proteins (Enzymes)” section of this chapter]. Furthermore, if enzymes that catalyze the reactions of a metabolic pathway are oriented sequentially, so that the product of one reaction is released in very close proximity to the next enzyme for which it is a substrate, the velocity of the overall pathway will be greatly enhanced. Evidence indicates that such an arrangement does in fact exist among the enzymes that participate in glycolysis.

Possibly all metabolic pathways occurring in the cytoplasmic matrix are influenced by its structural arrangement. The separation or association of metabolic pathways (or both) is important in regulating metabolism. This topic is covered more fully in Chapter 8. Metabolic pathways of particular significance that occur in the

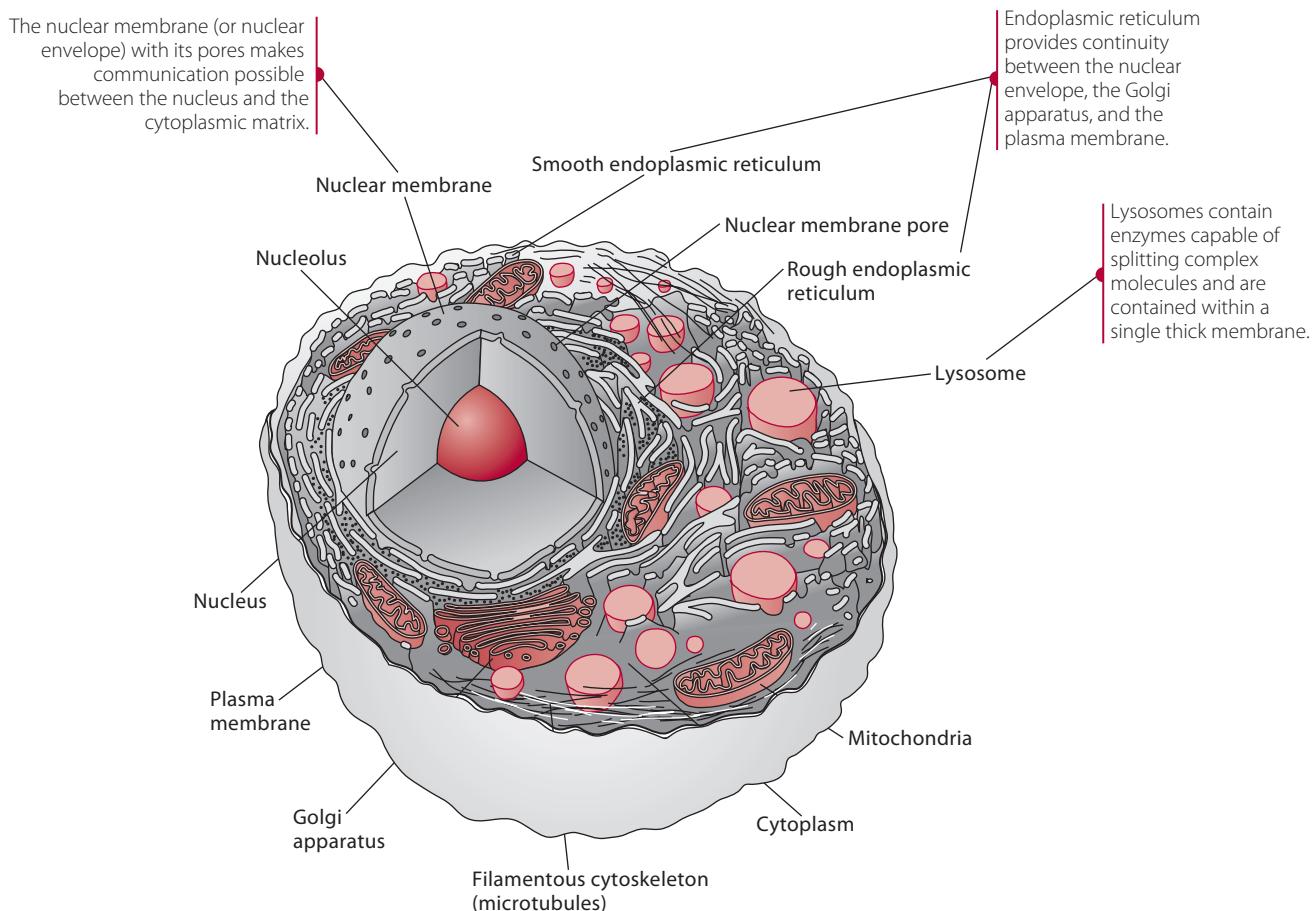


Figure 1.5 Three-dimensional depiction of a typical mammalian liver cell.

cytoplasmic matrix and that might be affected by its structure include:

- glycolysis
- hexose monophosphate shunt (pentose phosphate pathway)
- glycogenesis and glycogenolysis
- fatty acid synthesis, including the production of nonessential, unsaturated fatty acids

Normal intracellular communication among all cellular components is vital for cell activation and survival. The importance of the microtubular network is evidenced by its function to support and interconnect cellular components. The network also helps components communicate.

The cytoplasmic matrix of eukaryotic cells contains a number of organelles, enclosed in the bilayer membrane. Each of these components is described briefly in the following sections. Figures 1.1 and 1.5 show these organelles.

MITOCHONDRION

The **mitochondria** are the primary sites of oxygen use in the cell and are responsible for most of the metabolic

energy (adenosine triphosphate, or ATP) produced in cells. The size and shape of the mitochondria in different tissues vary according to the function of the tissue. In muscle tissue, for example, the mitochondria are held tightly among the fibers of the contractile system. In the liver, however, the mitochondria have fewer restraints, appear spherical, and move freely through the cytoplasmic matrix.

Mitochondrial Membrane

The mitochondrion consists of a matrix or interior space surrounded by a double membrane (Figures 1.6 and 1.7).

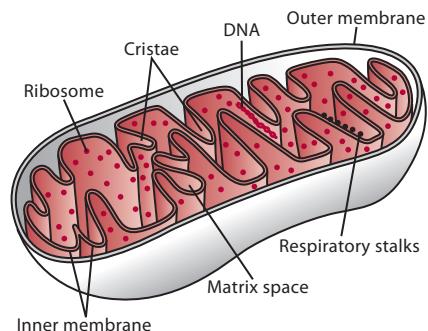


Figure 1.6 The mitochondrion.

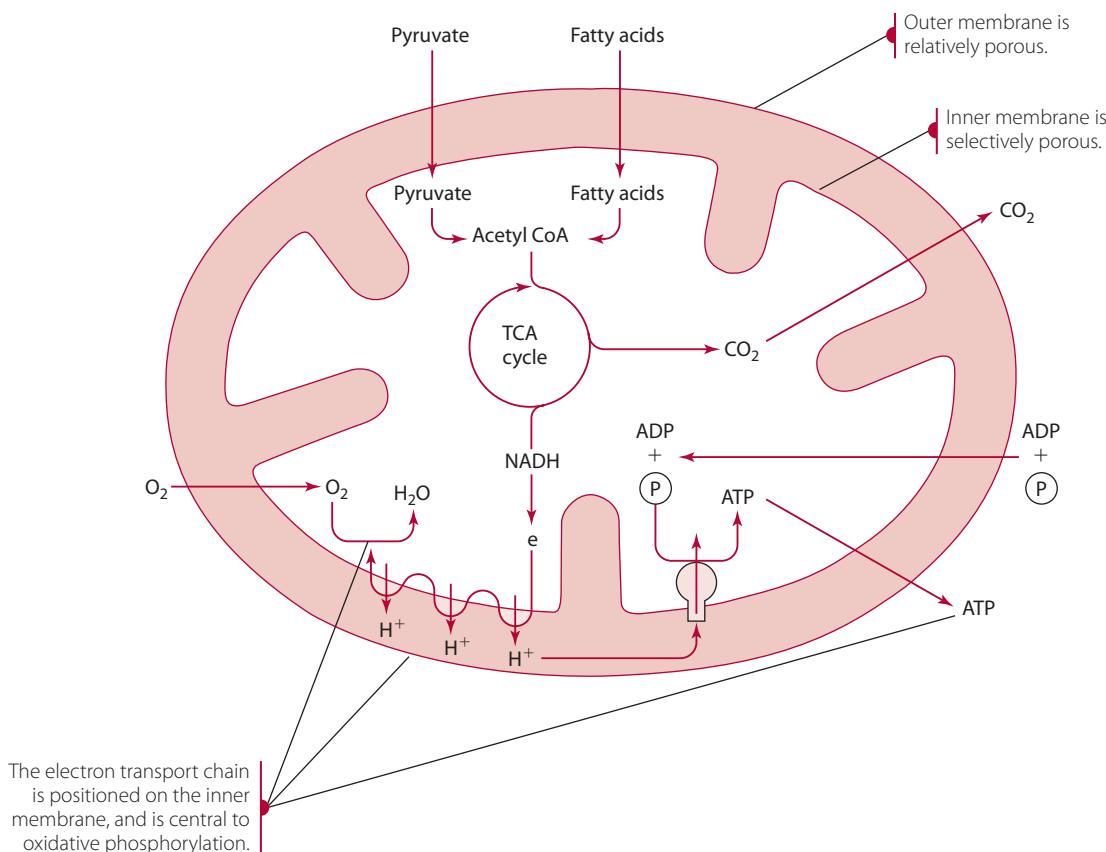


Figure 1.7 Overview of a cross section of the mitochondria.

The mitochondrial outer membrane is relatively porous, whereas the inner membrane is selectively permeable, serving as a barrier between the cytoplasmic matrix and the mitochondrial matrix. The inner membrane has many invaginations, called the cristae, which increase the surface area of the inner membrane in which all the components of the electron transport chain are embedded.

The electron transport (respiratory) chain is central to the process of **oxidative phosphorylation**, the mechanism by which most cellular ATP is produced. The components of the electron transport chain carry electrons and hydrogens during catalytic oxidation of nutrient molecules by enzymes in the mitochondrial matrix. The details of this process are described more fully in Chapter 3. Briefly, the mitochondria carry out the flow of electrons through the electron transport chain. This electron flow is strongly exothermic, and the energy released is used in part for ATP synthesis, an endothermic process. Molecular oxygen is ultimately, but indirectly, the oxidizing agent in these reactions. The function of the **electron transport chain** is to couple the energy released by nutrient oxidation to the formation of ATP. The chain components are precisely positioned within the inner mitochondrial membrane, an important feature of the mitochondria, because it brings the oxidizable products released in the matrix into close proximity with molecular oxygen.

Figure 1.7 shows the flow of major reactants into and out of the mitochondrion.

Mitochondrial Matrix

Among the metabolic enzyme systems functioning in the mitochondrial matrix are those that catalyze the reactions of the TCA cycle and fatty acid oxidation (Chapter 5). Other enzymes are involved in the oxidative decarboxylation and carboxylation of pyruvate (Chapter 3) and in certain reactions of amino acid metabolism (Chapter 6).

The mitochondria reproduce by dividing in two. Although the nucleus contains most of the cell's deoxyribonucleic acid (DNA), the mitochondrial matrix contains a small amount of DNA and a few ribosomes, so limited protein synthesis occurs within the mitochondrion. The genes contained in mitochondrial DNA, unlike those in the nucleus, are inherited only from the mother [3]. The primary function of mitochondrial genes is to code for proteins vital to producing ATP [2]. Most of the enzymes operating in the mitochondrion, however, are coded by nuclear DNA and synthesized on the rough endoplasmic reticulum (RER) in the cytoplasm. They are then incorporated into existing mitochondria.

All cells in the body, with the exception of the erythrocyte, possess mitochondria. The erythrocyte disposes of its mitochondria during the maturation process and

then must depend solely on the energy produced through anaerobic mechanisms, primarily glycolysis.

NUCLEUS

The cell nucleus is the largest of the organelles and, because of its DNA content, initiates and regulates most cellular activities. Surrounding the nucleus is the **nuclear envelope**. The nuclear envelope is composed of two bilayer membranes (an inner and an outer membrane) that are dynamic structures (Figure 1.5). The dynamic nature of these membranes makes communication possible between the nucleus and the cytoplasmic matrix and allows a continuous channel between the nucleus and the endoplasmic reticulum. At various intervals the two membranes of the nuclear envelope fuse, creating pores in the envelope (Figure 1.5). The nucleus and the microtubules of the cytoskeleton appear to be interdependent. The polymerization and the intracellular distribution of the microtubules are controlled by nucleus-based activities. Clusters of proteins on the outer nuclear membrane are centers of these activities. These clusters, called microtubule organization centers (MTOCs), begin polymerizing and organizing the microtubules during mitosis. A review of MTOC activity has been published [4,5].

The matrix held within the nuclear envelope contains molecules of DNA that encode the cell's genetic information

plus all the enzymes. The matrix also contains the minerals necessary for the activity of the nucleus. Condensed regions of the chromatin within the nuclear envelope, called **nucleoli**, contain not only DNA and its associated alkaline proteins (histones) but also considerable amounts of RNA (ribonucleic acid). This particular RNA is believed to give rise to the microsomal RNA (i.e., RNA associated with the endoplasmic reticulum).

Encoded within the nuclear DNA of the cell are thousands of genes that direct the synthesis of proteins. Each gene codes for a single specific protein. The **cell genome** is the entire set of genetic information: all of the DNA within the cell. Barring mutations that may arise in the DNA, daughter cells, produced from a parent cell by mitosis, possess the identical genomic makeup of the parent. The process of DNA replication enables the DNA to be precisely copied at the time of mitosis.

After the cell receives a signal that protein synthesis is needed, protein biosynthesis occurs in phases called transcription, translation, and elongation (Figure 1.8). Each phase requires DNA activity, RNA activity, or both. These phases, together with replication, are reviewed briefly in this chapter, but the scope of this subject is large; interested readers should consult a current, comprehensive biochemistry text for a more thorough treatment of protein biosynthesis [6].

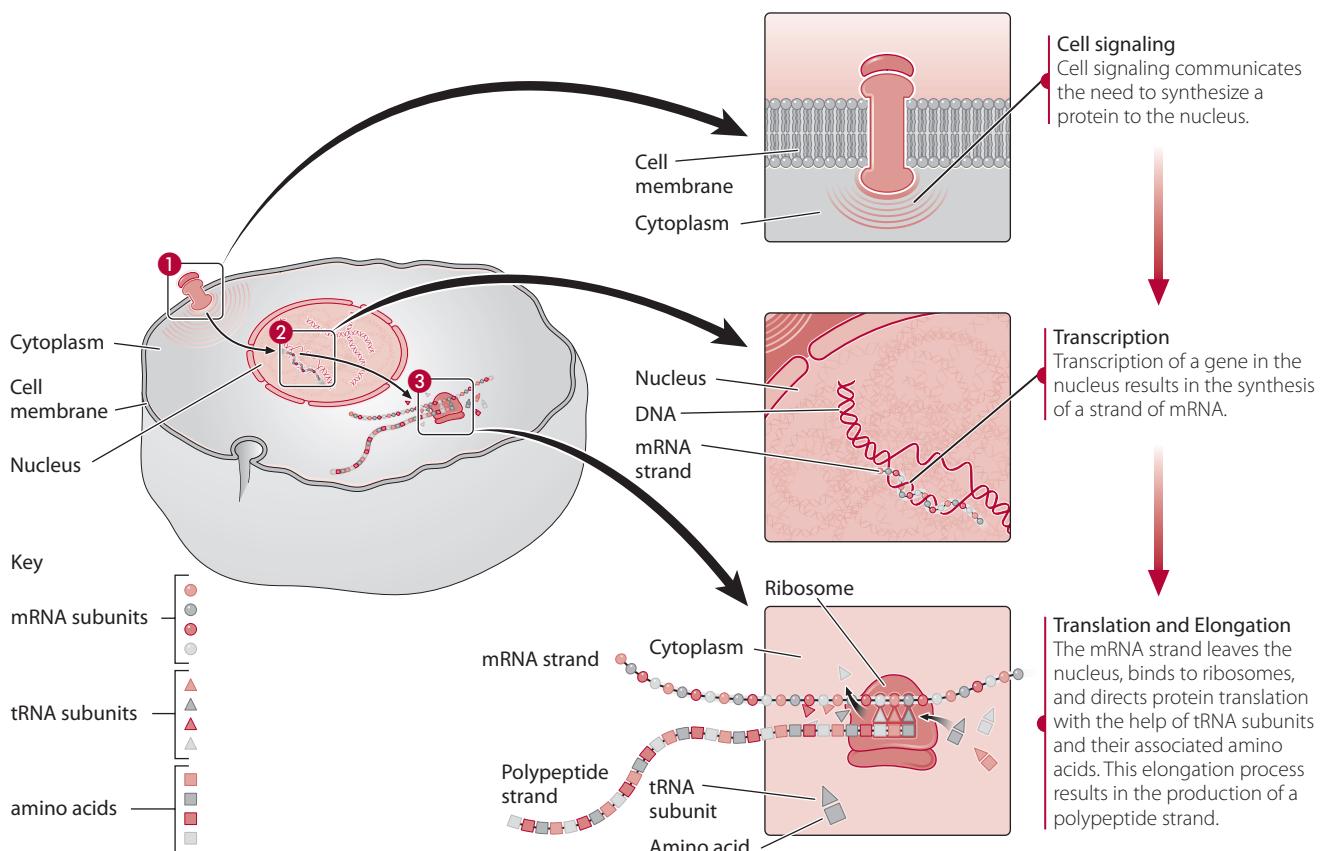


Figure 1.8 Steps of protein synthesis.

Nucleic Acids

Nucleic acids (DNA and RNA) are formed from repeating units called **nucleotides**, sometimes referred to as nucleotide bases or just bases. Structurally, they consist of a nitrogenous core (either purine or pyrimidine), a pentose sugar (ribose in RNA, deoxyribose in DNA), and phosphate. Five different nucleotides are contained in the structures of the nucleic acids: Adenylic acid and guanylic acid are purines, and cytidylic acid, uridylic acid, and thymidylic acid are pyrimidines. The nucleotides are more commonly referred to by their nitrogenous base core only—namely, adenine, guanine, cytosine, uracil, and thymine, respectively. For convenience, particularly in describing the sequence of the polymeric nucleotides in a nucleic acid, the single-letter abbreviations are most often used. Adenine (A), guanine (G), and cytosine (C) are common to both DNA and RNA, whereas uracil (U) is unique to RNA, and thymine (T) is found only in DNA. When two strands of nucleic acids interact with each other, as occurs in replication, transcription, and translation, bases in one strand pair specifically with bases in the second strand: A always pairs with T or U, and G pairs with C, in what is called **complementary base pairing**, Figure 1.9.

The nucleotides are connected by phosphates esterified to hydroxyl groups on the pentose—that is, deoxyribose or ribose—component of the nucleotide. The carbon atoms of the pentoses are assigned prime (') numbers for

identification. The phosphate group connects the 3' carbon of one nucleotide with the 5' carbon of the next nucleotide in the sequence. The 3' carbon of the latter nucleotide in turn is connected to the 5' carbon of the next nucleotide in the sequence, and so on. Therefore, nucleotides are attached to each other by 3', 5' diester bonds. The ends of a nucleic acid chain are called either the free 3' end or the free 5' end, meaning that the hydroxyl groups at those positions are not attached by phosphate to another nucleotide.

Cell Replication

Cell replication involves the synthesis of a daughter DNA molecule identical to the parental DNA. At cell division, the cell must copy its genome with a very high degree of fidelity. Each strand of the DNA molecule acts as a template for synthesizing a new strand. Figures 1.8 and 1.9 illustrate replication by base pairing and show the formation of the two new strands. The DNA molecule consists of two large strands of nucleic acid that are intertwined to form a double helix. During cell division the two unravel, with each forming a template for synthesizing a new strand through complementary base pairing. Incoming nucleotide bases first pair with their complementary bases in the template and then are connected through phosphate diester bonds by DNA polymerase, an enzyme. The end result of the **replication** process is two new DNA chains that join with the two chains from the parent molecule to produce two new DNA molecules. Each new DNA molecule is therefore identical in base sequence to the parent, and each new cell of a tissue consequently carries within its nucleus identical information to direct its functioning. The two strands in the DNA double helix are antiparallel, which means that the free 5' end of one strand is connected to the free 3' end of the other. With this process, a cell is able to copy or replicate its genes before it passes them on to the daughter cell. Although errors sometimes occur during replication, mechanisms exist that correct or repair mismatched or damaged DNA. Refer to a biochemistry text [6] for details.

Cell Transcription

Transcription is the process by which the genetic information (through the sequence of base pairs) in a single strand of DNA makes a specific sequence of bases in an mRNA chain (Figure 1.8). Transcription proceeds continuously throughout the entire life cycle of the cell. In the process, various sections of the DNA molecule unravel, and one strand—called the **sense strand**—serves as the template for synthesizing messenger RNA (mRNA). The genetic code of the DNA is transcribed into mRNA through complementary base pairing, as in DNA replication, except that the purine adenine (A) pairs with the pyrimidine uracil (U) instead of with thymine (T). **Genes** are composed of critically sequenced base pairs along the entire length of the DNA strand that is being transcribed.

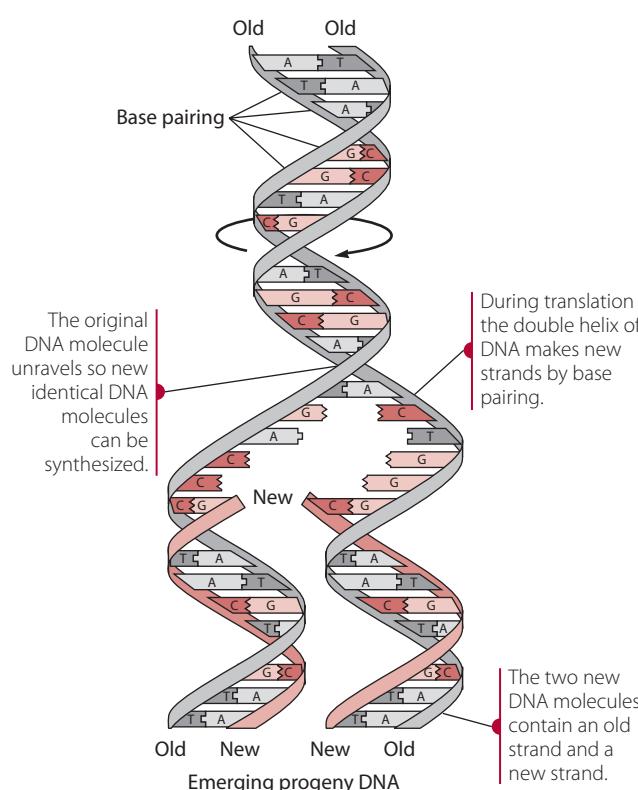


Figure 1.9 DNA replication.

A gene, on average, is just over 1,000 base pairs in length, compared with the nearly 5 million (5×10^6) base pair length of typical chromosomal DNA chains. Although these figures provide a rough estimate of the number of genes per transcribed DNA chain, not all the base pairs of a gene are transcribed into functional mRNA.

Genes translate certain regions of nucleotide sequences into complementary mRNA sequences that do not code for a protein product. These segments, called **introns** (intervening sequences), have to be removed from the mRNA before it is translated into protein (see the “Translation” section of this chapter). Enzymes excise the introns from the newly formed mRNA, and the ends of the functional, active mRNA segments are spliced together in a process called posttranscriptional processing. The gene segments that get both transcribed and translated into the protein product, called **exons** (expressed sequences), require no posttranscriptional processing.

Translation

Translation is the process by which genetic information in an mRNA molecule specifies the sequence of amino acids in the protein product. After the mRNA is synthesized in the nucleus (Figure 1.8), the mRNA is exported into the cytoplasmic matrix, where it is attached to the ribosomes of the rough endoplasmic reticulum (RER) or to the free-standing polysomes (polyribosomes, Figure 1.4). On the ribosomes, the transcribed genetic code is used to bring amino acids into a specific sequence that produces a protein with a clearly delineated function.

The genetic code for specifying the amino acid sequence of a protein resides in the mRNA in the form of three-base sequences called **codons**. Each codon codes for a single amino acid. Although a given amino acid may have several codons (for example, the codons CUU, CUC, CUA, and CUG all code for the amino acid leucine), codons can code for only one amino acid. Each amino acid has one or more transfer RNAs (tRNA), which deliver the amino acid to the mRNA for peptide synthesis. The three-base sequences of the tRNA attach to the codons by complementary base pairing.

Amino acids are first activated by ATP at their carboxyl end and then transferred to their specific tRNAs that bear the anticodon complementary to each amino acid's codon. For example, because codons that code for leucine are sequenced CUU, CUC, CUA, or CUG, the only tRNAs to which an activated leucine can be attached would need to have the anticodon sequence GAA, GAG, GAU, or GAC. The tRNAs then bring the amino acids to the mRNA situated at the protein synthesis site on the ribosomes. After the amino acids are positioned according to codon-anticodon association, peptide bonds are formed between the aligned amino acids in a process called **elongation** (Figure 1.8). Elongation extends the polypeptide chain of the protein product by translation.

Each incoming amino acid is connected to the end of the growing peptide chain with the free carboxyl group (C-terminal end) by formation of further peptide bonds. New amino acids are incorporated until all the codons (corresponding to one gene) of the mRNA have been translated. At this point, the process stops abruptly, signaled by a “nonsense” codon that does not code for any amino acid. The completed protein dissociates from the mRNA. After translation, the new protein may require some chemical, structural, or spatial modification to attain its active form.

ENDOPLASMIC RETICULUM AND GOLGI APPARATUS

The **endoplasmic reticulum (ER)** is a network of membranous channels pervading the cytoplasm and providing continuity between the nuclear envelope, the Golgi apparatus, and the plasma membrane. This structure, therefore, is a mechanism for communication from the innermost part of the cell to its exterior (Figures 1.1 and 1.5).

The ER cannot be separated from the cell as an entity by laboratory preparation. During mechanical homogenization, the structure of the ER is disrupted and reforms into small spherical particles called microsomes. The ER is classified as either rough (granular) or smooth (agranular). The granularity or lack of granularity is determined by the presence or absence of ribosomes. Rough endoplasmic reticulum (RER), so named because it is studded with ribosomes, abounds in cells where protein synthesis is a primary function. Smooth endoplasmic reticulum (SER) is found in most cells; however, because it is the site of synthesis for a variety of lipids, it is more abundant in cells that synthesize steroid hormones (e.g., the adrenal cortex and gonads) and in liver cells, which synthesize fat transport molecules (the lipoproteins). In skeletal muscle, SER is called **sarcoplasmic reticulum** and is the site of the calcium ion pump, a necessity for the contractile process.

Ribosomes associated with RER are composed of ribosomal RNA (rRNA) and structural protein. All proteins to be secreted (or excreted) from the cell or destined to be incorporated into an organelle membrane in the cell are synthesized on the RER. The clusters of ribosomes (i.e., polyribosomes or polysomes) that are freestanding in the cytoplasm are also the synthesis site for some proteins. All proteins synthesized in polyribosomes in the cytosol (the liquid portion of the cytoplasm) remain within the cytoplasmic matrix or are incorporated into an organelle.

Located on the RER of liver cells is a system of enzymes very important in detoxifying and metabolizing many different drugs. This enzyme complex consists of a family of cytochromes, called the P450 system, that functions along with other enzymes. The P450 system is particularly active in oxidizing drugs, but because its action results in the

simultaneous oxidation of other compounds as well, the system is collectively referred to as the mixed-function oxidase system. Lipophilic substances—for example, the steroid hormones and numerous drugs—can be made hydrophilic by oxidation, reduction, or hydrolysis, thereby enabling them to be excreted easily in the bile or urine. This system is discussed further in Chapter 5.

The **Golgi apparatus** functions closely with the ER in trafficking and sorting proteins synthesized in the cell. The Golgi apparatus is particularly prominent in neurons and secretory cells. The Golgi apparatus consists of four to eight membrane-enclosed, flattened cisternae that are stacked in parallel (Figures 1.1 and 1.5). The Golgi cisternae are often referred to as “stacks” because of this arrangement. Tubular networks have been identified at either end of the Golgi stacks:

- The *cis*-Golgi network is a compartment that accepts newly synthesized proteins coming from the ER.
- The *trans*-Golgi network is the exit site of the Golgi apparatus. It sorts proteins for delivery to their next destination [7].

Proteins to be transferred into the Golgi apparatus are synthesized on the RER. The polypeptide forms within the Golgi apparatus as synthesis occurs. The Golgi apparatus is the site for membrane differentiation and the development of surface specificity. For example, the polysaccharide moieties of mucopolysaccharides and of the membrane glycoproteins are synthesized and attached to the polypeptide during its passage through the Golgi apparatus. Such an arrangement allows for the continual replacement of cellular membranes, including the plasma membrane.

The ER is a quality-control organelle in that it prevents proteins that have not achieved normal tertiary or quaternary structure from reaching the cell surface. The ER can retrieve or retain proteins destined for residency within the ER, or it can target proteins for delivery to the *cis*-Golgi compartment. Retrieved or exported protein “cargo” is coated with protein complexes called coatomers, abbreviated COPs (coat proteins). Some coatomers are structurally similar to the clathrin coat of endocytic vesicles and are described later in this chapter. The choice of what is retrieved or retained by the ER and what is exported to the Golgi apparatus is probably mediated by signals that are inherent in the terminal amino acid sequences of the proteins in question. Certain amino acid sequences of cargo proteins are thought to interact specifically with certain coatomers [8].

The membrane-bound compartments of the ER and the Golgi apparatus are interconnected by transport vesicles, in which cargo proteins are moved from compartment to compartment. The vesicles leaving a compartment are formed by a budding and pinching off of the compartment membrane, and the vesicles then fuse with the

membrane of the target compartment. The specificity of vesicle-membrane interactions has been the focus of considerable research [8].

Secretion from the cell of products such as proteins can be either constitutive or regulated. If secretion follows a constitutive course, the secretion rate remains relatively constant, uninfluenced by external regulation. Regulated secretion, as the name implies, is affected by regulatory factors, and therefore its rate is changeable.

LYSOSOMES AND PEROXISOMES

Lysosomes and **peroxisomes** are cell organelles packed with enzymes. Whereas the lysosomes serve as the cell’s digestive system, the peroxisomes perform some specific oxidative catabolic reactions. Lysosomes are particularly large and abundant in cells that perform digestive functions—for example, the macrophages and leukocytes. Approximately 36 powerful enzymes capable of splitting complex substances such as proteins, polysaccharides, nucleic acids, and phospholipids are held within the confines of a single thick membrane. The lysosome, just like a protein synthesized for excretion, is believed to develop through the combined activities of the ER and the Golgi apparatus. The result is a very carefully packaged group of lytic enzymes (Figures 1.1 and 1.5).

The membrane surrounding these catabolic enzymes has the capacity for very selective fusion with other vesicles so that **catabolism** (or digestion) may occur as necessary. Wastes produced by this process can be removed from the cells by exocytosis. Important catabolic activities performed by the lysosomes include participation in **phagocytosis**, in which foreign substances taken up by the cell are digested or rendered harmless. An example of digestion by lysosomes is their action in the proximal tubules of the kidney. Lysosomes of the proximal tubule cells are believed to digest the albumin absorbed by endocytosis from the glomerular filtrate. Lysosomal phagocytosis protects against invading bacteria and is part of the normal repair process following a wound or an infection.

A second catabolic activity of lysosomes is **autolysis**, in which intracellular components, including organelles, are digested following degeneration or cellular injury. Autolysis also can serve as a survival mechanism for the cell as a whole. Digesting dispensable intracellular components can provide the cell with nutrients necessary to fuel functions essential to life. The mitochondrion is an example of an organelle whose degeneration requires autolysis. It is estimated that the mitochondria of liver cells must be renewed approximately every 10 days.

Another catabolic activity of the lysosomes is bone resorption, an essential process in the normal modeling of bone. Lysosomes of the osteoclasts promote mineral dissolution and collagen digestion, both of which are necessary actions in bone resorption and in regulating calcium and

phosphorus homeostasis. Lysosomes, with their special membrane and numerous catabolic enzymes, also function in hormone secretion and regulation. Their role in the secretion of the thyroid hormones is particularly important (see Chapter 12).

In the early 1960s, the peroxisomes were first recognized as separate intracellular organelles. These small bodies are believed to originate by “budding” from the SER. The peroxisomes are similar to the lysosomes in that they are bundles of enzymes surrounded by a single membrane. Rather than having digestive action, however, the enzymes within the peroxisomes are catabolic oxidative enzymes. Although the mitochondrial matrix is the major site where fatty acids are oxidized to acetyl coenzyme A (acetyl CoA), the peroxisomes can carry out a similar series of reactions. Acetyl CoA produced in peroxisomes cannot be further oxidized for energy at that site, however, and must be transported to the mitochondria for oxidation through the TCA cycle.

Peroxisomes are also the site for certain reactions of amino acid catabolism. Some oxidative enzymes involved in these pathways catalyze the release of hydrogen peroxide (H_2O_2) as an oxidation product. Because H_2O_2 is a very reactive chemical that could cause cellular damage if not promptly removed or converted, H_2O_2 -releasing reactions are segregated within these organelles. The enzyme catalase, present in large quantities in the peroxisomes, degrades the potentially harmful H_2O_2 into water and molecular oxygen. Other enzymes in the peroxisomes are important in detoxifying reactions. Particularly important is the oxidation of ethanol to acetaldehyde.

Cellular Proteins

Proteins synthesized on the cell's free polyribosomes remain within the cell to perform their specific structural, digestive, regulatory, or other functions. Among the more interesting areas of biomolecular research has been determining how newly synthesized protein finds its way from the ribosomes to its intended destination. At the time of synthesis, signal sequences direct proteins to their appropriate target compartment. These targeting sequences, located at the N-terminus of the protein, are generally cleaved (though not always) when the protein reaches its destination. Interaction between the signal sequences and specific receptors located on the various membranes permits the protein to enter its designated membrane or become incorporated into the designated organelle.

A long list of metabolic diseases is attributed to a deficiency of, or the inactivity of, certain enzymes. Tay-Sachs disease, phenylketonuria, maple syrup urine disease, and the lipid and glycogen storage diseases are a few well-known

examples. As a result of research on certain mitochondrial proteins, it is believed that in at least some cases the enzymes are not necessarily inactive or deficient but rather fail to reach their correct destination [9–11].

Several cellular proteins are of particular interest to the health science student:

- **receptors**, proteins that modify the cell's response to its environment
- **transport proteins**, proteins that regulate the flow of nutrients into and out of the cell
- **enzymes**, the catalysts for the hundreds of biochemical reactions taking place in the cell

RECEPTORS AND INTRACELLULAR SIGNALING

Receptors are highly specific proteins located in the plasma membrane facing the exterior of the cell. Bound to the outer surface of these specific proteins are oligosaccharide chains, which are believed to act as recognition markers. Membrane receptors act as attachment sites for specific external stimuli such as hormones, growth factors, antibodies, lipoproteins, and certain nutrients and examples are shown in Figures 1.10 and 1.11. These molecular stimuli, which bind specifically to receptors, are called **ligands**. Receptors are also located on the membrane of cell organelles; less is known about these receptors, but they appear to be glycoproteins necessary for correctly positioning newly synthesized cellular proteins.

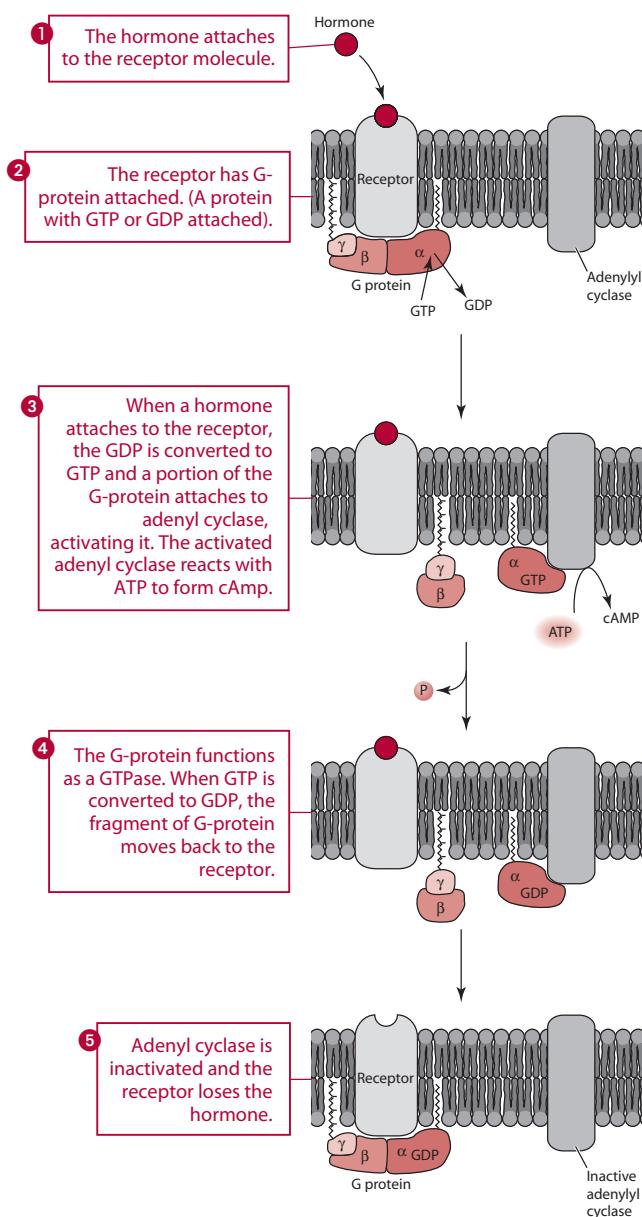
Although most receptor proteins are probably integral membrane proteins, some may be peripheral. In addition, receptor proteins can vary widely in their composition and mechanism of action. Although the composition and mechanism of action of many receptors have not yet been determined, at least three distinct types of receptors are known to exist:

- those that bind the ligand stimulus and convert it into an internal signal that alters behavior of the affected cell
- those that function as ion channels
- those that internalize their stimulus intact

Examples of these three types of receptors follow.

Internal Chemical Signal

The internal chemical signal most often produced by a stimulus-receptor interaction is 3', 5'-cyclic adenosine monophosphate (cyclic AMP, or cAMP). It is formed from adenosine triphosphate (ATP) by the enzyme adenyl cyclase. Cyclic AMP is frequently referred to as the second messenger in the stimulation of target cells by hormones. Figure 1.10 presents a model for the ligand-binding action of receptors, which leads to production of the internal signal cAMP. As shown in the figure, the stimulated receptor reacts with the guanosine triphosphate (GTP)-binding



protein (G-protein), which activates adenyl cyclase, triggering production of cAMP from ATP. G-protein is a trimer protein with three subunits (designated α , β , and γ). The α -subunit combines with GDP or GTP and has GTPase activity. Attachment of a hormone to the receptor stimulates the exchange of GDP for GTP. The GTP binding causes the trimers to disassociate and the α unit to associate with an effector protein, adenyl cyclase. A single hormone-binding site can produce many cAMP molecules.

The mechanism of action of cAMP signaling within the cell is complex, but it can be viewed briefly as follows: cAMP is an activator of protein kinases. **Protein kinases** are enzymes that phosphorylate (add phosphate groups to)

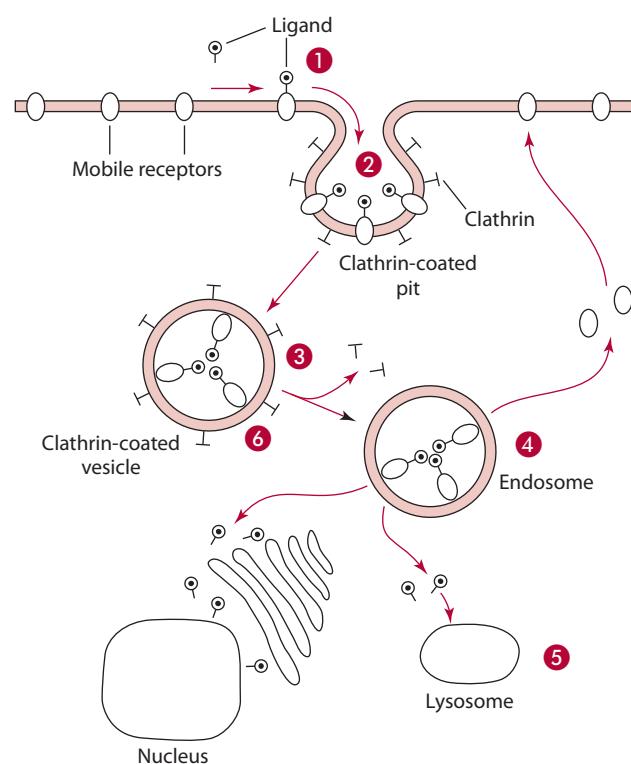


Figure 1.11 Internalization of a stimulus into a cell via its receptor.

other enzymes and, in doing so, convert the enzymes from inactive forms into active forms. In some cases, the phosphorylated enzyme is the inactive form. Protein kinases that can be activated by cAMP contain two subunits, a catalytic and a regulatory subunit. In the inactive form of the kinase, the two subunits are bound in such a way that the catalytic portion of the molecule is inhibited sterically by the presence of the regulatory subunit. Phosphorylation of the enzyme by cAMP causes the subunits to dissociate, thereby freeing the catalytic subunit, which regains its full catalytic capacity.

Ion Channel

Receptors can also act as ion channels in stimulating a cell. In some cases, the binding of the ligand to its receptor causes a voltage change, which then becomes the signal for an appropriate cellular response. Such is the case when the neurotransmitter acetylcholine is the stimulus. The receptor for acetylcholine appears to function as an ion channel in response to voltage change. Stimulation by acetylcholine signals the channels to open, allowing sodium (Na^+) ions to pass through an otherwise impermeable membrane [12].

Internalization Stimulus

The internalization of a stimulus into a fibroblast by way of its receptor is illustrated in Figure 1.11. Receptors that perform in such a manner exist for a variety of biologically

active molecules, including the hormones insulin and triiodothyronine. Low-density lipoproteins (LDLs) are taken up by certain cells in much the same fashion (see Chapter 5), except that their receptors, rather than being mobile, are already clustered in coated pits. These coated pits, vesicles formed from the plasma membrane, are coated with several proteins, among which clathrin is primary. A coated pit containing the receptor with its ligand soon loses the clathrin coating and forms a smooth-walled vesicle. This vesicle delivers the ligand into the depths of the cell and then is recycled, along with the receptor, into the plasma membrane. If the endocytotic process is for scavenging, the ligand (perhaps a protein) is not used by the cell but instead undergoes lysosomal degradation, as shown in Figure 1.11 and exemplified by the endocytosis of LDL.

The reaction of a fibroblast to changes in blood glucose levels is a good example of cellular adjustment to the existing environment that is made possible through receptor proteins. When blood glucose levels are low, muscular activity leads to release of the hormone epinephrine by the adrenal medulla. Epinephrine becomes attached to its receptor protein on the fibroblast, thereby activating the receptor and causing it to stimulate the G protein and adenyl cyclase, which catalyzes the formation of cAMP from ATP. Then cAMP initiates a series of enzyme phosphorylation modifications, as described earlier in this section, which result in the phosphorolysis of glycogen to glucose 1-phosphate for use by the fibroblast.

In contrast, when blood glucose levels are elevated, the hormone insulin, secreted by the β -cells of the pancreas, reacts with its receptors on the fibroblast and is transported into the cell by receptor-mediated endocytosis (Figure 1.11). Insulin allows diffusion of glucose into the cell by increasing the number of glucose receptors in the cell membrane, which in turn promote diffusion of glucose by its transport protein. Glucose transporters are covered in Chapter 3. The hormone itself is degraded within the cell [1].

Many intracellular chemical messengers are known other than those cited as examples in this section [13]. Listed here, along with cAMP, are several additional examples:

- cyclic AMP
- cyclic GMP
- Ca^{+2}
- inositol triphosphate
- diacyl glycerol
- fructose 2,6-bisphosphate

TRANSPORT PROTEINS

The cell produces many different types of proteins. So far, this chapter has covered the synthesis of structural

proteins. We will now focus on the functional proteins, which include transport proteins and catalytic proteins. We will then examine some of the factors that turn on or turn off the activity of specific proteins. These factors are the basis for nutritional control over the expression of certain genes.

Transport proteins regulate the flow of substances (including nutrients) into and out of the cell. Transport proteins may function by acting as carriers (or pumps), or they may provide protein-lined passages (pores) through which water-soluble materials of small molecular weight may diffuse. Figure 1.3 shows the integral and peripheral proteins of the cell membrane that function as transport proteins.

The active transport protein that has been studied most is the sodium (Na^+) pump. The Na^+ pump is essential not only to maintain ionic and electrical balance but also for intestinal and renal absorption of certain key nutrients (e.g., glucose and certain amino acids). These nutrients move into the epithelial cell of the small intestine against a concentration gradient, necessitating both a carrier and a source of energy, both of which are provided by the Na^+ pump.

The proposed mechanism by which glucose is actively absorbed is called symport because it is a simultaneous transport of two compounds (Na^+ and glucose) in the same direction. A transport protein with two binding sites binds both Na^+ and glucose. The attachment of Na^+ to the carrier increases the transport protein's affinity for glucose. Sodium, because it is moving down a concentration gradient created by energy released through Na^+/K^+ adenosine triphosphatase (ATPase), is able to carry along with it glucose that is moving up a concentration gradient. When Na^+ is released inside the cell, the carrier's affinity for glucose is decreased, and glucose also can be released into the cell. Na^+/K^+ -ATPase then "pumps" the Na ions back out of the cell. The sodium pump is illustrated in Figure 1.12.

Na^+/K^+ -ATPase works by first combining with ATP in the presence of Na^+ on the inner surface of the cell membrane. The enzyme then is phosphorylated by the breakdown of ATP to adenosine diphosphate (ADP) and consequently is able to move three Na ions out of the cell. On the outer surface of the cell membrane, ATPase becomes dephosphorylated by hydrolysis in the presence of K ions and then is able to return two K ions to the cell. The term *pump* is used because the Na and K ions are both transported across the membrane against their concentration gradients. This pump is responsible for most of the active transport in the body.

Transport of glucose and amino acids into the epithelial cells of the intestinal tract is active in that the carriers needed for their transport are dependent on the concentration gradients achieved by the action of Na^+/K^+ -ATPase at the basolateral membrane. The activity of Na^+/K^+ -ATPase is the major energy demand of the body at rest. The process of facilitated (non-energy

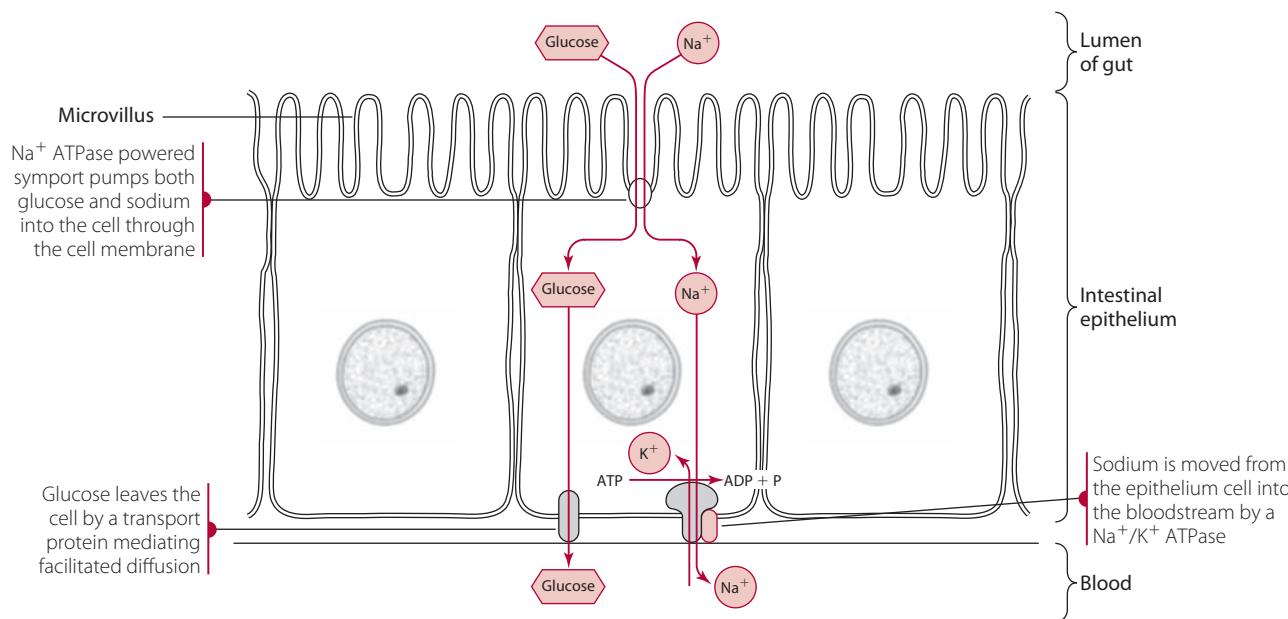


Figure 1.12 The active transport of glucose.

dependent) transport is also a very important mechanism for regulating the flow of nutrients into the cell. It is used broadly across a wide range of cell types. Proteins involved in this function are often called transporters; probably the most thoroughly studied of these are the glucose transporters, discussed in Chapter 3.

CATALYTIC PROTEINS (ENZYMES)

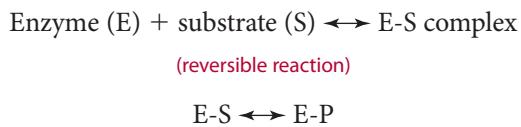
Enzymes are proteins that are distributed throughout all cellular compartments. Enzymes that are components of the cellular membranes are usually found on the inner surface of the membranes. Exceptions are the digestive enzymes: isomaltase, the disaccharidases (lactase, sucrase, and maltase), and certain peptidases located on the brush border of the epithelial cells lining the small intestine. These enzymes are described more fully in Chapter 2. Membrane-associated enzymes are found distributed throughout the cell organelles, with the greatest concentration found in the mitochondria. As mentioned earlier, the enzymes of the electron transport chain, where energy transformation occurs, are located within the inner membrane of the mitochondria.

Metabolic processes occurring in the cells are governed by enzymes that have been synthesized on the cell's RER under the direction of mRNA that was produced from nuclear DNA. The functional activity of most enzymes, however, depends not only on the protein portion of the molecule but also on a nonprotein prosthetic group or coenzyme. If the nonprotein group is an organic compound, it usually contains a chemically modified B-complex vitamin. Commonly, however, the prosthetic group is inorganic (i.e., metal ions such as Mg, Zn, Cu, Mn, or Fe).

Enzymes have an active center that possesses a high specificity. This specificity means that a substrate must fit perfectly into the specific contours of the active center. The velocity of an enzyme-catalyzed reaction increases as the concentration of the substrate that is available to the enzyme increases. However, this relationship applies only to a concentration of substrate that is less than the concentration that "saturates" the enzyme. At saturation levels of substrate, the enzyme molecule functions at its maximum velocity (V_{max}), and the occurrence of a still higher concentration of substrate cannot increase the velocity further.

K_m , or the Michaelis constant, is a useful parameter that aids in establishing how enzymes react in the living cell. K_m represents the concentration of a substrate that is found in an occurring reaction when the reaction is at one-half its maximum velocity. If an enzyme has a high K_m value, then an abundance of substrate must be present to raise the rate of reaction to half its maximum; in other words, the enzyme has a low affinity for its substrate. An example of an enzyme with a high K_m is glucokinase, an enzyme operating in the liver cells. Because glucose can diffuse freely into the liver, the fact that glucokinase has a high K_m is very important to blood glucose regulation. The low affinity of glucokinase for glucose prevents too much glucose being removed from the blood during periods of fasting. Conversely, when the glucose load is high—for example, following a high-carbohydrate meal—excess glucose can still be converted by glucokinase. The liver glucokinase does not function at its maximum velocity when glucose levels are in the normal range. The enzyme therefore can be thought of as a protection against high cellular concentrations of glucose.

The nature of enzyme catalysis can be described by the following reactions:



The substrate activated by combination with the enzyme is converted into an enzyme-product complex through rearrangement of the substrate's ions and atoms:



The product is released, and the enzyme is free to react with more of the substrate.

Reversibility

Most biochemical reactions are reversible, meaning that the same enzyme can catalyze a reaction in both directions. The extent to which a reaction can proceed in a reverse direction depends on several factors, the most important of which are the relative concentrations of substrate (reactant) and product and the differences in energy content between reactant and product. In instances when a very large disparity in energy content or concentration exists between reactant and product, the reaction can proceed in only one direction. Such a reaction is unidirectional rather than reversible. This topic is discussed later in this chapter. In unidirectional reactions, the same enzyme cannot catalyze in both directions. Instead, a different enzyme is required to catalyze the reverse direction of the reaction. Comparing glycolysis with gluconeogenesis allows us to see how unidirectional reactions may be reversed by introducing a different enzyme.

Simultaneous reactions, catalyzed by various multienzyme systems or pathways, constitute cellular metabolism. Enzymes are compartmentalized within the cell and function in sequential chains. A good example of a multienzyme system is the TCA cycle located in the mitochondrial matrix. Each sequential reaction is catalyzed by a different enzyme, and some reactions are reversible, whereas others are unidirectional. Although some reactions in almost any pathway are reversible, it is important to understand that removing one of the products drives the reaction toward forming more of that product. Removing (or using) the product, then, becomes the driving force that causes reactions to proceed primarily in the desired direction.

Regulation

A very important aspect of nutritional biochemistry is the regulation of metabolic pathways. Anabolic and catabolic reactions must be kept in a balance appropriate for life (and perhaps growth) of the organism. Regulation involves primarily the adjustment of the catalytic activity of certain

participating enzymes. This regulation occurs through three major mechanisms:

- covalent modification of enzymes through hormone stimulation
- modulation of allosteric enzymes
- increase in enzyme concentration by induction

Covalent Modification The first of these mechanisms, covalent modification of enzymes, is usually achieved by the addition of or hydrolytic removal of phosphate groups to or from the enzyme. This is the mechanism involving cAMP and protein kinase activation covered in the “Internal Chemical Signal” section of this chapter. An example of covalent modification of enzymes is the regulation of glycogenesis and glycogenolysis.

Allosteric The second important regulatory mechanism is that exerted by certain unique enzymes called allosteric enzymes. The term *allosteric* refers to the fact that these enzymes possess an allosteric or specific “other” site besides the catalytic site. Specific compounds, called modulators, can bind to these allosteric sites and profoundly influence the activity of these regulatory enzymes. Modulators may be positive (i.e., causing an increase in enzyme activity), or they may exert a negative effect (i.e., inhibit activity). Modulating substances are believed to alter the activity of the allosteric enzymes by changing the conformation of the polypeptide chain or chains of the enzyme, thereby altering the binding of its catalytic site with the intended substrate. Negative modulators are often the end products of a sequence of reactions. As an end product accumulates above a certain critical concentration, it can inhibit, through an allosteric enzyme, its own further production.

An excellent example of an allosteric enzyme is phosphofructokinase in the glycolytic pathway. Glycolysis gives rise to pyruvate, which is decarboxylated and oxidized to acetyl CoA and enters the TCA cycle by combination with oxaloacetate to form citrate. Citrate is a negative modulator of phosphofructokinase. Therefore, an accumulation of citrate causes glycolysis inhibition by regulating phosphofructokinase. In contrast, an accumulation of AMP or ADP, which indicates that ATP is depleted, signals the need for additional energy in the cell in the form of ATP. AMP or ADP therefore modulates phosphofructokinase positively. The result is an active glycolytic pathway that ultimately leads to the formation of more ATP through the TCA cycle–electron transport chain connection.

Induction The third mechanism of enzyme regulation, *enzyme induction*, creates changes in the concentration of certain inducible enzymes. Inducible enzymes are adaptive, meaning that they are synthesized at rates dictated by cellular circumstances. In contrast, constitutive enzymes, which

are synthesized at a relatively constant rate, are uninfluenced by external stimuli. Induction usually occurs through the action of certain hormones, such as the steroid hormones and the thyroid hormones, and is exerted through changes in the expression of genes encoding the enzymes. Dietary changes can elicit the induction of enzymes necessary to cope with the changing nutrient load. This regulatory mechanism is relatively slow, however, compared to the first two mechanisms discussed, which exert effects in terms of seconds or minutes.

The reverse of induction is the blockage of enzyme synthesis by blocking the formation of the mRNA of specific enzymes. This regulation of translation is one of the means by which small molecules, reacting with cellular proteins, can exert their effect on enzyme concentration and the activity of metabolic pathways.

Specific examples of enzyme regulation are described in subsequent chapters that deal with metabolism of the major nutrients. It should be noted at this point, however, that *enzymes targeted for regulation catalyze essentially unidirectional reactions*. In every metabolic pathway, at least one reaction is essentially irreversible, exergonic, and enzyme limited. That is, the rate of the reaction is limited only by the activity of the enzyme catalyzing it. Such enzymes are frequently the regulatory enzymes, capable of being stimulated or suppressed by one of the mechanisms described. Logically, an enzyme catalyzing a reaction reversibly at near equilibrium in the cell cannot be regulatory, because its up or down regulation would affect its forward and reverse activities equally. This effect, in turn, would not accomplish the purpose of regulation, which is to stimulate the rate of the metabolic pathway in one direction to exceed the rate of the pathway in the reverse direction.

Examples of Enzyme Types

Enzymes participating in cellular reactions are located throughout the cell in both the cytoplasmic matrix (cytoplasm) and the various organelles. The location of specific enzymes depends on the site of the metabolic pathways or metabolic reactions in which those enzymes participate. Enzyme classification, therefore, is based on the type of reaction catalyzed by the various enzymes. Enzymes fall within six general classifications:

- **Oxidoreductases** (dehydrogenases, reductases, oxidases, peroxidases, hydroxylases, and oxygenases) are enzymes that catalyze all reactions in which one compound is oxidized and another is reduced. Examples of oxidoreductases are the enzymes found in the electron transport chain located on the inner membrane of the mitochondria. Other examples are the cytochrome P450 enzymes located on the ER of liver cells.
- **Transferases** are enzymes that catalyze reactions not involving oxidation and reduction, in which a functional

group is transferred from one substrate to another. Included in this group of enzymes are transketolase, transaldolase, transmethylase, and the transaminases. The transaminases (α -amino transferases), which figure so prominently in protein metabolism, fall under this classification and are located primarily in the mitochondrial matrix.

- **Hydrolases** (esterases, amidases, peptidases, phosphatases, and glycosidases) are enzymes that catalyze cleavage of bonds between carbon atoms and some other kind of atom by adding water. Digestive enzymes fall within this classification, as do those enzymes contained within the lysosome of the cell.
- **Lyases** (decarboxylases, aldolases, synthetases, cleavage enzymes, deaminases, nucleotide cyclases, hydrases or hydratases, and dehydratases) are enzymes that catalyze cleavage of carbon-carbon, carbon-sulfur, and certain carbon-nitrogen bonds (peptide bonds excluded) without hydrolysis or oxidation-reduction. Citrate lyase, which frees acetyl CoA for fatty acid synthesis in the cytoplast, is a good example of an enzyme belonging to this classification.
- **Isomerases** (isomerases, racemases, epimerases, and mutases) are enzymes that catalyze the interconversion of optical or geometric isomers. Phosphohexose isomerase, which converts glucose 6-phosphate to fructose 6-phosphate in glycolysis (occurring in the cytoplast), exemplifies this particular class of enzyme.
- **Ligases** are enzymes that catalyze the formation of bonds between carbon and a variety of other atoms, including oxygen, sulfur, and nitrogen. Forming bonds catalyzed by ligases requires energy that usually is provided by hydrolysis of ATP. A good example of a ligase is acetyl CoA carboxylase, which is necessary to initiate fatty acid synthesis in the cytoplast. Through the action of acetyl CoA carboxylase, a bicarbonate ion (HCO_3^-) is attached to acetyl CoA to form malonyl CoA, the initial compound in starting fatty acid synthesis.

PRACTICAL CLINICAL APPLICATION OF CELLULAR ENZYMES

All of the hundreds of enzymes present in the human body are synthesized intracellularly, and most of them function within the cell in which they were formed. These are the enzymes responsible for catalyzing the myriad of metabolic reactions occurring in each cell. As explained in the “Cellular Proteins” section of this chapter, proteins are directed to specific locations within the cell or excreted from the cell after they are synthesized on the ribosomes. Many of the enzymes are secreted from the cell in an inactive form, and are rendered active in the extracellular fluids where they function. Examples of secreted enzymes

are the digestive proteases and other hydrolases formed in the cells of the pancreas and then secreted into the lumen of the small intestine. Other secreted enzymes, called plasma-specific enzymes, function in the bloodstream. Examples include the enzymes involved in the blood-clotting mechanism.

Diagnostic enzymology focuses on intracellular enzymes, which, because of a problem within the cell structure, escape from the cell and ultimately express their activity in the serum. By measuring the serum activity of the released enzymes, both the site and the extent of the cellular damage can be determined. If the site of the damage is to be determined with reasonable accuracy, the enzyme being measured must exhibit a relatively high degree of organ or tissue specificity. For example, an enzyme having a concentration many times greater in hepatocytes in the liver than its concentration in other tissues could potentially be a marker for liver damage should its serum activity increase. The rate at which intracellular enzymes enter the bloodstream is based on the rates at which enzymes leak from cells and rates at which the enzyme is produced. Enzyme production can be altered by increased synthesis within the cell or by an increase in the number of cells producing the enzyme.

Intracellular enzymes normally are retained within the cell by the plasma membrane. The plasma membrane is metabolically active, and its integrity depends on the cell's energy consumption and therefore its nutritive status. Any process that impairs the cell's use of nutrients can compromise the structural integrity of the plasma membrane. Membrane failure can also arise from mechanical disruption, such as would be caused by a viral attack on the cell. Damage to the plasma membrane is manifested as leakiness and eventual cell death, allowing unimpeded passage of substances, including enzymes, from intracellular to extracellular compartments.

Factors contributing to cellular damage and resulting in abnormal egress of cellular enzymes include the following events:

- tissue ischemia, (**ischemia** refers to an impairment of blood flow to a tissue or part of a tissue; it deprives affected cells of oxygen and oxidizable nutrients)
- tissue necrosis
- viral attack on specific cells
- damage from organic chemicals such as alcohol and organophosphorus pesticides
- hypoxia (inadequate intake of oxygen)

Increases in blood serum concentrations of cellular enzymes can be good indicators of even minor cellular damage because the intracellular concentration of enzymes is hundreds or thousands of times greater than in blood and also because enzyme assays are extremely sensitive.

Conditions for Diagnostic Suitability

Not all intracellular enzymes are valuable in diagnosing damage to the cells in which they are contained. Several conditions must be met for the enzyme to be suitably diagnostic:

- *The enzyme must have a sufficiently high degree of organ or tissue specificity.* Suppose an enzyme is widely distributed among organ or tissue systems. Although an abnormal increase in the activity of this enzyme in serum does indicate a pathological process with cellular damage, it cannot precisely identify the site of the damage. An example is lactate dehydrogenase (LDH). LDH activity is widely distributed among cells of the heart, liver, skeletal muscle, erythrocytes, platelets, and lymph nodes. Therefore, elevated serum activity of LDH can hardly be a specific marker for tissue pathology. In practice, however, LDH does have diagnostic value if it is first separated into its five different isoenzyme forms and each is quantified individually. Each isoform is more organ-specific than total LDH. For instance, one is primarily associated with heart muscle, and another is associated with liver cells.
- *A steep concentration gradient of enzyme activity must exist between the interior and exterior of the cells under normal conditions.* If this condition were not true, small increases in serum activity would not be detectable. Examples of enzymes that are in compliance with this requirement and that have been useful over the years as disease markers are prostatic acid phosphatase, with a prostate cell to serum concentration ratio of 103:1, and alanine aminotransferase, with a hepatocyte to serum ratio of 104:1. These enzymes have been useful in diagnosing prostatic disease (primarily carcinoma) and viral hepatitis, respectively.
- *The enzyme must function in the cytoplasmic compartment of the cell.* If the enzyme is compartmentalized within an organelle such as the nucleus or mitochondrion, its leakage from the cell is impeded even in the event of significant damage to the plasma membrane. An example of an enzyme that does not comply with this condition is the mitochondrial enzyme ornithine carbamoyl transferase, which functions within the urea cycle. Although the enzyme adheres rigidly to the two previous conditions (i.e., it is strictly liver specific, and its cell to serum ratio is as high as 105:1), it provides little value in diagnosing hepatic disease.
- *The enzyme must be stable for a reasonable period of time in the vascular compartment.* Isocitrate dehydrogenase has an extremely high activity in heart muscle. Yet, following the resultant damage of a myocardial infarction, the released enzyme is rapidly inactivated upon entering the bloodstream, thereby becoming indeterminable.

Increased Production Factors

The most common cause of increased production of an enzyme, resulting in a spike in its serum concentration,

is malignant disease. Substances that occur in body fluids as a result of malignant disease are called tumor markers. A tumor marker may be produced by the tumor itself or by the host, in response to a tumor.

In addition to enzymes and isoenzymes, other forms of tumor markers include hormones, oncofetal protein antigens such as carcinoembryonic antigen (CEA), and products of oncogenes. **Oncogenes** are mutated genes that encode abnormal, mitosis-signaling proteins that cause unchecked cell division.

Products of malignant cells, such as intracellular enzymes, exhibit a predictably increased rate of synthesis because of the nature of the disease process. If the proliferating cells of the tumor retain their capacity to synthesize the enzyme, the gross output of the enzyme is markedly elevated. Furthermore, the enzyme can be released into the systemic circulation as a result of tumor necrosis or the change in permeability of the plasma membrane of the malignant cells.

Although tumor markers are present in higher quantities in cancer tissue or blood from cancer patients than in benign tissue or the blood of normal subjects, few markers are specific for the organ in which the tumor is located, because most enzymes are not unique to a specific organ. A possible exception is prostate-specific antigen (PSA).

PSA is a proteolytic enzyme produced almost exclusively by the prostate gland. Its value as a tumor marker is further enhanced by the fact that metastasized malignant prostate cells produce nearly 10 times as much PSA as do normal prostate cells. A significant rise in serum PSA concentration therefore may signal that a tumor has metastasized to other sites in the body, suggesting a different therapeutic approach. Though other causes exist for an increase in PSA levels besides cancer, PSA has become a valuable screening and diagnostic tool. Prostate cancer is the leading cause of death among older men.

Table 1 offers a list of enzymes that have been used successfully as indicators of organ or tissue pathology.

Table 1 Diagnostically Important Enzymes

Enzyme Significance	Principal Source	Principal Clinical
Acid phosphatase	Prostate, erythrocytes	Carcinoma of prostate
Alanine amino transferase	Liver, skeletal muscle, heart	Hepatic parenchymal cell disease
Aldolase	Skeletal muscle, heart	Muscle diseases
Amylase	Pancreas, salivary glands	Pancreatitis, carcinoma of pancreas
Cholinesterase	Liver	Organophosphorus insecticide poisoning, hepatic parenchymal cell disease
Creatine kinase (CK-2 isoform)	Heart, skeletal muscle	Myocardial infarction
Gamma glutamyl transferase	Liver, kidney	Alcoholism, hepatobiliary disease
Prostate-specific antigen (PSA)	Prostate	Carcinoma of prostate

The principal sources of the enzymes and the clinical significance of their serum determination are also included.

Apoptosis

Dying is said to be a normal part of living. So it is with the cell. Like every living thing, a cell has a well-defined life span, after which its structural and functional integrity diminishes and it is removed by other cells through phagocytosis.

As cells die, they are replaced by new cells that are continuously being formed by cell mitosis. However, both daughter cells formed in the mitotic process do not always enjoy the full life span of the parent. If they did, the number of cells, and consequently tissue mass, could increase inordinately. Therefore, one of the two cells produced by mitosis generally is programmed to die before its sister. In fact, most dying cells are already doomed at the time they are formed. Those targeted for death are usually smaller than their surviving sisters, and their phagocytosis begins even before the mitosis generating them is complete. The processes of cell division and cell death must be carefully regulated in order to generate the proper number of cells during development. Once cells mature, the appropriate number of cells must be maintained. The mechanism by which naturally occurring cell death arises has been subjected to intense research in recent years [14–16]. The mechanisms involving cell death and those reactions that control them are important in the development of certain cancers and in immunological reactions.

PROGRAMMED DEATH

Many terms have been used to describe naturally occurring cell death. It is now most commonly referred to as *programmed cell death*, to distinguish it from pathological cell death, which is not part of any normal physiological process. The emergent term describing programmed cell death is **apoptosis**, a word borrowed from the Greek meaning to “fall out.”

POTENTIAL MECHANISMS

Several mechanisms result in apoptotic cell death. This is an area of active research, and much has been learned about those factors that initiate the process and those that inhibit it. The details of the cell biology and biochemistry of apoptosis exceed the scope of this book. Many excellent reviews are available [14–17] for readers interested in a detailed description.

In mammalian cells, apoptosis is triggered by intra- and extracellular stimuli. The intracellular stimuli create DNA

damage on specific genes. This damage causes a release of proapoptotic factors from the mitochondria into the cytoplasm. The release of these factors is antagonized by proteins originating from specific genes. One of the proteins released from the mitochondria is cytochrome c [16]. This protein activates a group of cysteine protease enzymes called caspases. The initial caspases activates additional caspases. The enzymes are called caspases because they hydrolyze the peptide chain at the amino acid cysteine. This proteolytic process is described in Chapter 6. One of the factors activated was previously associated with oncogenesis. If cell death is prevented from occurring, a transformed cell can continue to grow rather than be destroyed, creating a tumor. Caspases are normally inactive in the cell and must be converted to an active form.

The release of cytochrome c from the mitochondrion into the cytoplasmic matrix is one factor that promotes apoptosis. Once cytochrome c has translocated to the cytoplasmic matrix, it activates the caspases. Protein designated Bcl-2 (B-cell lymphoma gene product) blocks the release of mitochondrial cytochrome c. By blocking the release of mitochondrial cytochrome c, Bcl-2 interferes with the apoptotic process. Bcl-2 is an integral membrane protein on the outer membrane of the mitochondrion.

Two observations are relevant:

- Bcl-2 prevents the efflux of cytochrome c from the mitochondrion to the cytoplasmic matrix.
- Genetic overexpression of Bcl-2 prevents cells from undergoing apoptosis in response to various stimuli.

Therefore, a possible role of Bcl-2 in preventing apoptosis is to block release of cytochrome c from the mitochondrion [16].

The extracellular pathway for apoptosis is initiated by the extracellular hormones or agonists that belong to the **tumor necrosis factor** (TNF) family. TNFs are **cytokines** that are very important in regulating metabolism. These compounds are discussed in Chapter 8. TNFs recognize and activate their corresponding receptors. Through a series of protein-protein interactions, they recruit specific adaptor proteins. TNFs trigger a cascade of active caspases and inhibit anti-apoptotic factors that lead to cell death.

One of the caspases activated by cytochrome c is a potent DNAase that cleaves the genome of the cell into fragments of approximately 180 base pairs. Dead cells are removed by phagocytosis.

Cell death appears to be activated by specific genes in dying cells. Genes designated Casp-9 and Apaf-1 must be expressed within dying cells for cell death to occur. The Casp-9 and Apaf-1 genes encode products (proteins) that activate cytotoxic activity, and they therefore must be tightly controlled to avoid damage to the wrong cells. A major control factor is a third gene, Bcl-2, which negatively regulates the Casp-9 and Apaf-1 genes. Mutations to Bcl-2, which inactivate the gene, have been shown to kill an animal

under study by causing the death of cells otherwise intended to survive [14–16].

Interestingly, many of the proteins released in the process of apoptosis are found in the mitochondria. Most have a specific role there. Only when they are released into the cytoplasm do they have a role in apoptosis.

Other mechanisms of cell death exist. One such mechanism is termed **oncrosis**. Oncrosis (from *onksos*, meaning swelling) is defined as a prelethal pathway leading to cell death accompanied by cellular swelling, organelle swelling, and increased membrane permeability. The process of oncrosis results in the depletion of cellular energy stores. Oncrosis may result from toxic substances or pathogens that interfere with ATP generation. This form of oncrosis differs from apoptosis that causes cell death without any inflammation process.

As stated earlier, the investigation into the mechanism of apoptosis is very active. The study of how cell death can be controlled has important disease implications. Investigating the death of cells in the heart following a myocardial infarction, the relationship between preventing apoptosis and oncogenesis, and cell death caused by pathological organisms may lead to future breakthroughs.

Biological Energy

The previous sections of this chapter provide some descriptive insight into the makeup of a cell, how it reproduces, and how large and small molecules are synthesized within a cell or move in or out of a cell. All of these activities require energy. The cell obtains this energy from small molecules transformed (oxidized) to provide chemical energy and heat. There needs to be a constant supply of small molecules, which is supplied by the nutrients in food. The next section covers some basics of energy needs in the cell.

Most of the processes that sustain life involve energy. Some processes use energy, and others release it. The term *energy* conjures an image of physical “vim and vigor,” the fast runner or the weight lifter straining to lift hundreds of pounds. This notion of energy is accurate insofar as the contraction of muscle fibers associated with mechanical work is an energy-demanding process, requiring adenosine triphosphate (ATP), the major storage form of molecular energy in the cell. Beyond the ATP required for physical exertion the living body has other, equally important, requirements for energy, including:

- the biosynthetic (anabolic) systems by which substances can be formed from simpler precursors
- active transport systems by which compounds or ions can be moved across membranes against a concentration gradient
- the transfer of genetic information

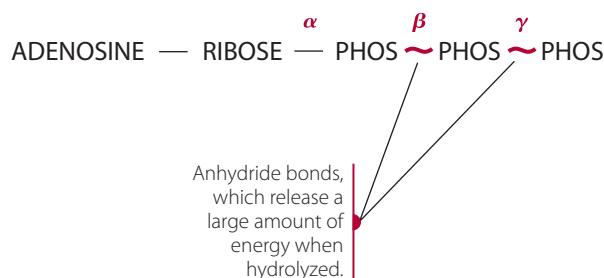


Figure 1.13 Adenosine triphosphate (ATP).

This section addresses the key role of energy transformation and heat production in using nutrients and sustaining life.

ENERGY RELEASE AND CONSUMPTION IN CHEMICAL REACTIONS

Energy used by the body is ultimately derived from the energy contained in the **macronutrients**—carbohydrate, fat, and protein (and alcohol). If this energy is released, it may simply be expressed as heat, as would occur in the combustion of flammable substances, or be preserved in the form of other chemical energy. Energy cannot be created or destroyed; it can only be transformed. Burning a molecule of glucose outside the body liberates heat, along with CO_2 and H_2O as products of combustion, as shown:



The metabolism of glucose to the same CO_2 and H_2O within the cell is nearly identical to that of simple combustion. The difference is that in metabolic oxidation a significant portion of the released energy is salvaged as chemical energy in the form of new, high-energy bonds. These bonds represent a usable source of energy for driving energy-requiring processes. Such stored energy is generally contained in phosphate anhydride bonds,

chiefly those of ATP (Figure 1.13). The analogy between the combustion and the metabolic oxidation of a typical nutrient (palmitic acid) is illustrated in Figure 1.14. The metabolic oxidation illustrated released 59% of the heat that was produced by the combustion and conserved about 40% of the chemical energy.

UNITS OF ENERGY

The unit of energy used throughout this text is the calorie, abbreviated cal. In the expression of the higher caloric values encountered in nutrition, the unit kilocalories (kcal) is often used: 1 kcal = 1,000 cal. The international scientific community and many scientific journals use another unit of energy, called the joule (J) or the kilojoule (kJ). Students of nutrition should be familiar with both units. Calories can easily be converted to joules by the factor 4.18:

$$1 \text{ cal} = 4.18 \text{ J}, \text{ or } 1 \text{ kcal} = 4.18 \text{ kJ}$$

To help you become familiar with both terms, this text primarily uses *calories* or *kilocalories*, followed by the corresponding values in joules or kilojoules in parentheses. Nutrition and the calorie have been closely linked over the years. However, although you may be more comfortable with the calorie and kilocalorie units, as a student of nutrition you should become familiar with joules and kilojoules.

Free Energy

The potential energy inherent in the chemical bonds of nutrients is released if the molecules undergo oxidation either through combustion or through oxidation within the cell. This energy is defined as **free energy** (*G*) if, on its release, it is capable of doing work at constant temperature and pressure—a condition that is met within the cell.

CO_2 and H_2O are the products of the complete oxidation of organic molecules containing only carbon,

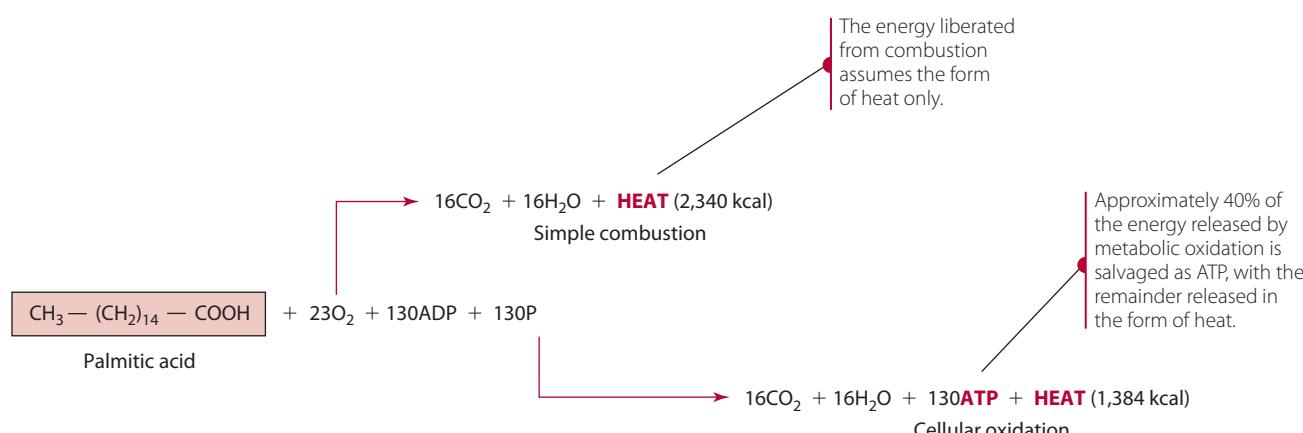


Figure 1.14 A comparison of the simple combustion and the metabolic oxidation of the fatty acid palmitate.

hydrogen, and oxygen, and they have an inherent free energy. The energy released in the course of oxidation of the organic molecules is in the form of either heat or chemical energy. The products have less free energy than do the original reactants. Because energy is neither created nor lost during the reaction, the total energy remains constant. Thus, the difference between the free energy in the products and that in the reactants in a given chemical reaction is a useful parameter for estimating the tendency for that reaction to occur. This difference is symbolized as follows:

$$G_{\text{products}} - G_{\text{reactants}} = \Delta G \text{ of the reaction}$$

where G is free energy and Δ is a symbol signifying change.

Exothermic and Endothermic Reactions

If the G value of the reactants is greater than the G value of the products, as in the case of the oxidation reaction, the reaction is said to be **exothermic**, or energy releasing, and the change in G (ΔG) is negative. In contrast, a positive ΔG indicates that the G value of the products is greater than that of the reactants, indicating that energy must be supplied to the system to convert the reactants into the higher-energy products. Such a reaction is called **endothermic**, or energy requiring.

Exothermic and endothermic reactions are sometimes referred to as downhill and uphill reactions, respectively, terms that help create an image of energy input and release. The free energy levels of reactants and products in a typical exothermic, or downhill, reaction can be likened to a boulder on a hillside that can occupy two positions, A and

B, as illustrated in Figure 1.15. As the boulder descends to level B from level A, energy capable of doing work is liberated, and the change in free energy is a negative value. The reverse reaction, moving the boulder uphill to level A from level B, necessitates an input of energy, or an endothermic process, and the change is a positive value. The quantity of energy released in the downhill reaction is precisely the same as the quantity of energy required for the reverse (uphill) reaction—only the sign of ΔG changes.

Activation Energy

Although exothermic reactions are favored over endothermic reactions in that they require no external energy input, they do not occur spontaneously. If they did, no energy-producing nutrients or fuels would exist throughout the universe, because they would all have transformed spontaneously to their lower energy level. A certain amount of energy must be introduced into reactant molecules to activate them to their **transition state**, a higher energy level or barrier at which the exothermic conversion to products can indeed take place. The energy that must be imposed on the system to raise the reactants to their transition state is called the **activation energy**. Refer again to the boulder-and-hillside analogy in Figure 1.15. The boulder does not spontaneously descend until the required activation energy can dislodge it from its resting place to the brink of the slope.

Cellular Energy

The cell derives its energy from a series of chemical reactions, each of which exhibits a free energy change. The reactions occur sequentially as nutrients are systematically oxidized ultimately to CO_2 and H_2O . Nearly all the reactions in the cell

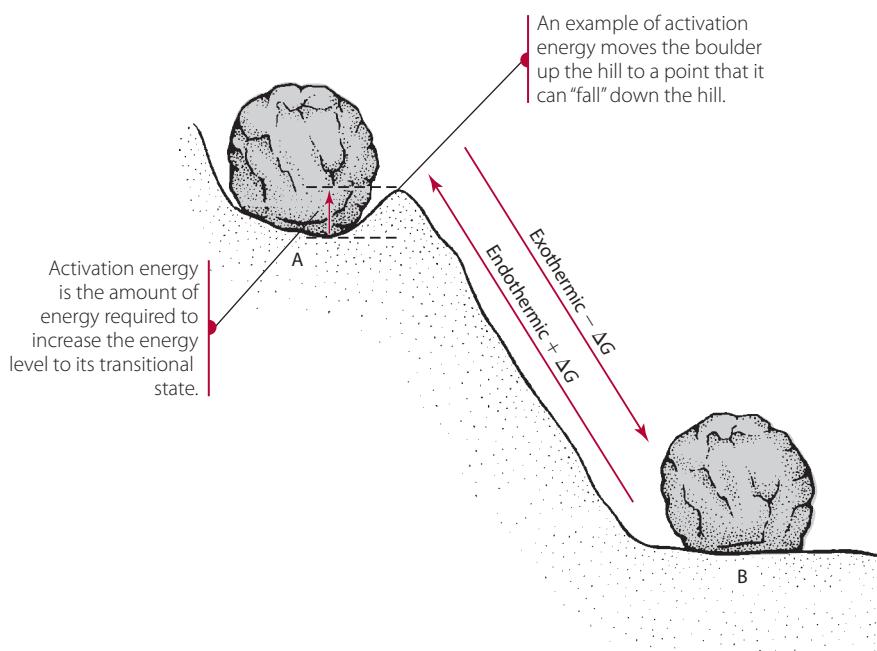


Figure 1.15 The uphill-downhill concept illustrating energy-releasing and energy-demanding processes.

are catalyzed by enzymes. Within a given catabolic pathway—for example, the oxidation of glucose to CO_2 and H_2O —some reactions may be energy consuming (have a $+ΔG$ for the reaction). However, energy-releasing (those with a $-ΔG$) reactions are favored, so the net energy transformation for the entire pathway has a $-ΔG$ and is exothermic.

Reversibility of Chemical Reactions

Most cellular reactions are reversible, meaning that an enzyme (E) that can catalyze the conversion of hypothetical substance A into substance B can also catalyze the reverse reaction, as shown:



Using the A, B interconversion as an example, let's review the concept of reversibility of a chemical reaction. In the presence of the specific enzyme E, substance A is converted to substance B. Initially, the reaction is unidirectional because only A is present. However, because the enzyme is also capable of converting substance B to substance A, the reverse reaction becomes significant as the concentration of B increases. From the moment the reaction is initiated, the amount of A decreases, while the amount of B increases to the point at which the rate of the two reactions becomes equal. At that point, the concentration of A and B no longer changes, and the system is said to be in equilibrium. Enzymes are only catalysts and do not change the equilibrium of the reaction. This concept is discussed more fully later. Whether the $\text{A} \rightarrow \text{B}$ reaction or the $\text{B} \rightarrow \text{A}$ reaction is energetically favored is indicated by the relative concentrations of A and B at equilibrium.

The equilibrium between reactants and products can be defined in mathematical terms and is called equilibrium constant (K_{eq}). K_{eq} is simply the ratio of the equilibrium concentration of product B to that of reactant A: $K_{\text{eq}} = [\text{B}]/[\text{A}]$. The [] signify the concentration. If the denominator ($[\text{A}]$) is very small, dividing it into a much larger number results in K_{eq} being large. $[\text{A}]$ will be small if most of A (the reactant) is converted to the product B. In other words, K_{eq} increases in value when the concentration of A decreases and that of B increases. If K_{eq} has a value greater than 1, substance B is formed from substance A, whereas a value of K_{eq} less than 1 indicates that at equilibrium A will be formed from B. An equilibrium constant equal to 1 indicates that no bias exists for either reaction. The K_{eq} of a reaction can be used to calculate the standard free energy change of the reaction.

Standard Free Energy Change

In order to compare the energy released or consumed in different reactions, it is convenient to define the free energy at standard conditions. Standard conditions are defined precisely: a temperature of 25°C (298 K); a pressure of

1.0 atm (atmosphere); and the presence of both the reactants and the products at their standard concentrations, namely 1.0 mol/L. The standard free energy change ($ΔG^0$) (the superscript zero designates standard conditions) for a chemical reaction is a constant for that particular reaction. The $ΔG^0$ is defined as the difference between the free energy content of the reactants and the free energy content of the products under standard conditions. Under such conditions, $ΔG^0$ is mathematically related to K_{eq} by the equation

$$ΔG^0 = -2.3 \text{ RT} \log K_{\text{eq}}$$

where R is the gas constant (1.987 cal/mol) and T is the absolute temperature, 298 K in this case. The factors 2.3, R, and T are constants, and their product is equal to $-2.3(1.987)(298)$, or $-1,362$ cal/mol. The equation therefore simplifies to

$$ΔG^0 = -1,362 \log K_{\text{eq}}$$

This topic is important in understanding the energetics of metabolic pathways, but you should refer to a biochemistry textbook for additional information on this subject.

Equilibrium Constant and Standard Free Energy Change

The equilibrium constant of a reaction determines the sign and magnitude of the standard free energy change. For example, referring once again to the $\text{A} \rightarrow \text{B}$ reaction, the logarithm of a K_{eq} value greater than 1.0 will be positive, and because it is multiplied by a negative number, the sign of $ΔG^0$ will be negative. We have established that the reaction $\text{A} \rightarrow \text{B}$ is energetically favored if $ΔG^0$ is negative. Conversely, the log of a K_{eq} value less than 1.0 would be negative, and when multiplied by a negative number the sign of $ΔG^0$ would be positive. The $ΔG^0$ in this case indicates that the formation of A from B ($\text{A} \leftarrow \text{B}$) is favored in the equilibrium.

Standard pH

For biological reactions, a standard pH has been defined. For most compartments in the body, the pH is near neutral; for biochemical reactions, a standard pH value of 7 is adopted by convention. For human nutrition, the free energy change of reactions is designated $ΔG^0$. This book uses this notation.

Nonstandard Physiological Conditions

Physiologically standard conditions do not often exist. The difference between standard conditions and non-standard conditions can explain why a reaction having a positive $ΔG^0$ can proceed exothermically ($-ΔG^0$) in the cell. For example, consider the reaction catalyzed by the enzyme triosephosphate isomerase (TPI) shown in Figure 1.16. This particular reaction occurs in the glycolytic pathway through which glucose is converted to pyruvate.

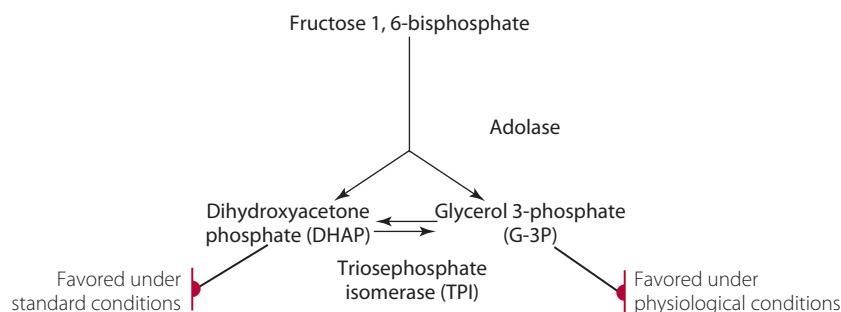


Figure 1.16 Example of a shift in the equilibrium by changing from standard conditions to physiological conditions.

(The chemical structures and the pathway are discussed in detail in Chapter 3). In the glycolytic pathway, the enzyme aldolase produces 1 mol each of dihydroxyacetone phosphate (DHAP) and glyceroldehyde 3-phosphate (G-3P) from 1 mol of fructose 1,6-bisphosphate. Let us focus on the reaction that TPI catalyzes, which is an isomerization between the two products of the aldolase reaction. As explained in Chapter 3, only the G 3-P is further degraded in the subsequent reactions of glycolysis. This circumstance results in a substantially lower concentration of the G 3-P metabolite than of DHAP.

For this reaction, two important conditions within the cell deviate from “standard conditions”: namely, the temperature is $\sim 37^\circ\text{C}$ (310 K), and neither the G 3-P nor DAHP are at 1.0 mol/L concentrations. The value of ΔG° for the reaction DHAP (reactant) \rightarrow G 3-P (product) is +1,830 cal/mol (+7,657 J/mol), indicating that under standard conditions the formation of DHAP is preferred over the formation of G 3-P. If we assume that the cellular concentration of DHAP is 50 times that of G 3-P because G 3-P is further metabolized ΔG° for the reaction is calculated to be equal to -577 cal/mol (-2,414 J/mol). The negative ΔG° shows that the reaction is favored to form G 3-P, as shown, despite the positive ΔG° for this reaction.

THE ROLE OF HIGH-ENERGY PHOSPHATE IN ENERGY STORAGE

The preceding section addressed the fundamental principle of free energy changes in chemical reactions and the fact that the cell obtains this chemical free energy through the catabolism of nutrient molecules. It also stated that this energy must somehow be used to drive the various energy-requiring processes and anabolic reactions so important in normal cell function. This section explains how ATP can be used as a universal source of energy to drive reactions. Examples of very high energy phosphate compounds are shown in Figure 1.17. Phosphoenolpyruvate and 1,3-diphosphoglycerate are components of the oxidative pathway of glucose (Chapter 3) and phosphocreatine is a storage form of high energy phosphate available to replenish ATP in muscle. The hydrolysis of the phosphate anhydride bonds of ATP can liberate the stored chemical energy when needed. ATP thus can be thought of as an energy reservoir, serving as the major linking intermediate between energy-releasing and energy-demanding chemical reactions in the cell. In nearly all cases, the energy stored in ATP is released by the enzymatic hydrolysis of the anhydride bond connecting the β - and γ -phosphates in the molecule (Figure 1.13). The products of this hydrolysis are adenosine diphosphate

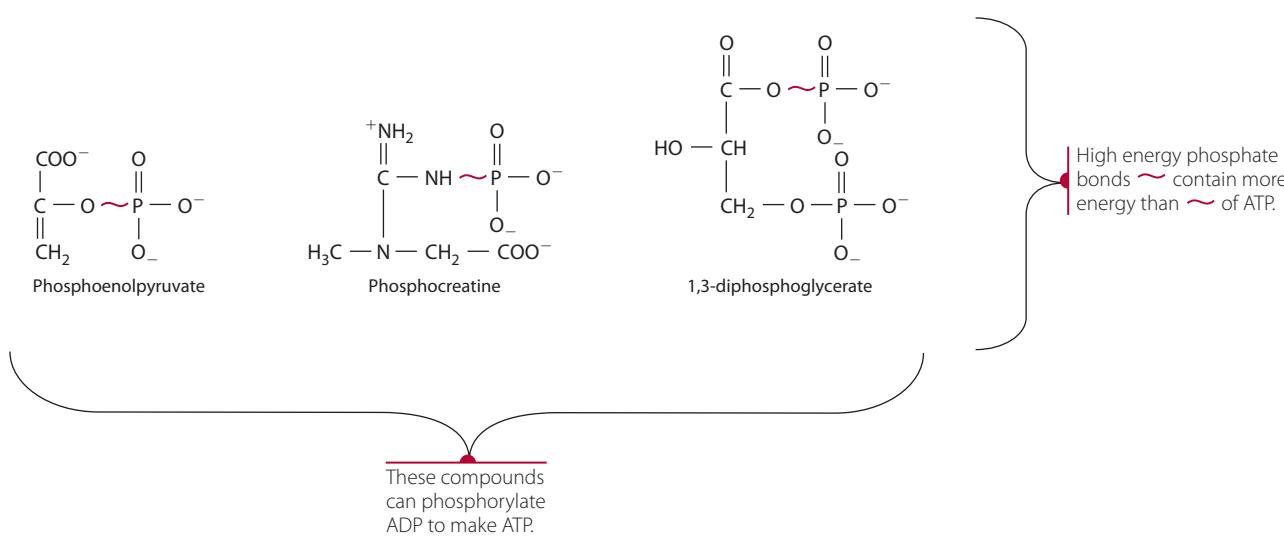


Figure 1.17 Examples of very high energy phosphate compounds.

(ADP) and inorganic phosphate (P_i). In certain instances, the free phosphate group is transferred to various acceptors, a reaction that activates the acceptors to higher energy levels. The involvement of ATP as a link between the energy-releasing and energy-requiring cellular reactions and processes is summarized in Figure 1.18.

COUPLED REACTIONS IN THE TRANSFER OF ENERGY

Some reactions require energy, and others yield energy. The coupling of these reactions makes it possible for a pathway to continue. The oxidation of glucose in the glycolysis pathway demonstrates the importance of coupled reactions in metabolism. An understanding of how chemical energy is transformed from macronutrients (the carbohydrate, protein, fat, and alcohol in food) to storage forms (such as ATP), and how the stored energy is used to synthesize needed compounds for the body, is fundamental to the study of human nutrition. These topics are covered in this section as well as throughout this book. The ΔG^0 value for the phosphate bond hydrolysis of ATP is intermediate between certain high-energy phosphate compounds and compounds that possess relatively low-energy phosphate esters. ATP's central position on the energy scale lets it serve as an intermediate carrier of phosphate groups. ADP can accept the phosphate groups from high-energy phosphate donor molecules and then, as ATP, transfer them to lower-energy receptor molecules. Two examples of this transfer are shown in Figure 1.19. By

receiving the phosphate groups, the acceptor molecules become activated to a higher energy level, from which they can undergo subsequent reactions such as entering the glycolysis pathway. The end result is the transfer of chemical energy from donor molecules through ATP to receptor molecules. The second example is the transfer of a P_i group from creatine phosphate to ADP. Creatine phosphate serves as a ready reservoir to renew ATP levels quickly, particularly in muscle.

If a given quantity of energy is released in an exothermic reaction, the same amount of energy must be added to the system for that reaction to be driven in the reverse direction. For example, hydrolysis of the phosphate ester bond of glucose 6-phosphate liberates 3,300 cal/mol (13.8 kJ/mol) of energy, and the reverse reaction, in which the phosphate is added to glucose to form glucose 6-phosphate, necessitates the input of 3,300 cal/mol (13.8 kJ/mol). These reactions can be expressed in terms of their standard free energy changes as shown in Figure 1.20. To phosphorylate glucose, the reaction must be coupled with the hydrolysis of ATP, which provides the necessary energy. The additional energy from the reaction is dissipated as heat.

The addition of phosphate to a molecule is called a phosphorylation reaction. It generally is accomplished by the enzymatic transfer of the terminal phosphate group of ATP to the molecule, rather than by the addition of free phosphate as suggested in Figure 1.20. The reverse reaction is hypothetical, designed only to illustrate the energy requirement for phosphorylation of the glucose molecule. In fact, the enzymatic phosphorylation of glucose by ATP is the first reaction glucose undergoes upon entering the cell. This reaction promotes glucose to a higher energy level, from which it may be indirectly incorporated into glycogen as stored carbohydrate or systematically oxidized for energy. Phosphorylation therefore can be viewed as occurring in two reaction steps:

1. hydrolysis of ATP to ADP and phosphate
2. addition of the phosphate to the substrate (glucose) molecule

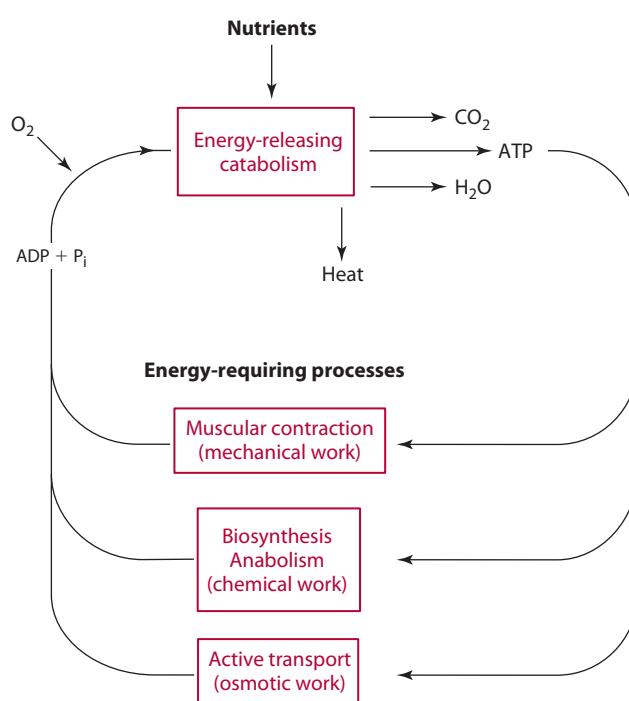


Figure 1.18 An illustration of how ATP is generated from the coupling of ADP and phosphate through the oxidative catabolism of nutrients and how it in turn is used for energy-requiring processes.

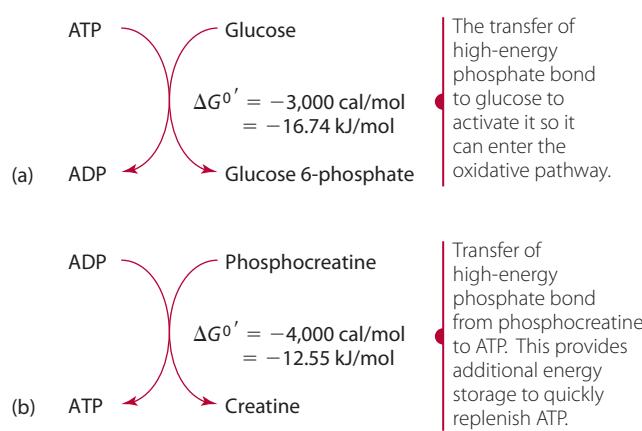
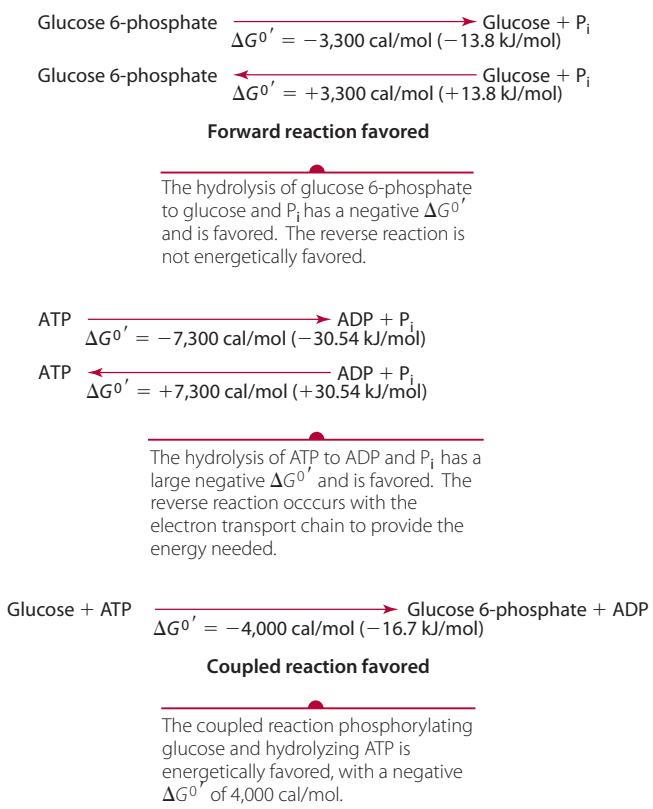


Figure 1.19 Examples of high-energy phosphate bonds being transferred.

**Figure 1.20** Exothermic reactions.

A net energy change for the two reactions coupled together is shown in Figure 1.20. The net ΔG^{0'} for the coupled reaction is −4,000 cal/mol (16.7 kJ/mol).

The significance of these coupled reactions cannot be overstated. They show that even though energy is consumed in the endothermic formation of glucose 6-phosphate from glucose and phosphate, the energy released by the ATP hydrolysis is sufficient to force (or drive) the endothermic reaction that “costs” only 3,300 cal/mol. The coupled reactions result in 4,000 cal/mol (16.7 kJ/mol) left over. The reaction is catalyzed by the enzyme hexokinase or glucokinase, both of which hydrolyze the ATP and transfer the phosphate group to glucose. The enzyme brings the ATP and the glucose into close proximity, reducing the activation energy of the reactants and facilitating the phosphate group transfer. The overall reaction, which results in activating glucose at the expense of ATP, is energetically favorable, as evidenced by its high, negative standard free energy change.

REDUCTION POTENTIALS

As we will see when we discuss the formation of ATP in Chapter 3, ATP is formed in the electron transport chain after the macronutrients are oxidized. To better understand these oxidations and reductions, you need to understand reduction potentials. The energy to

synthesize ATP becomes available following a sequence of individual reduction-oxidation (redox) reactions along the electron transport chain, with each component having a characteristic ability to donate and accept electrons. The released energy is used in part to synthesize ATP from ADP and phosphate. The tendency of a compound to donate and to receive electrons is expressed in terms of its **standard reduction potential**, E_{0'}. The more negative the values of E_{0'} are, the greater the ability of the compound to donate electrons, whereas increasingly positive values signify an increasing tendency to accept electrons. The reducing capacity of a compound (its tendency to donate H⁺ and electrons) can be expressed by the E_{0'} value of its half-reaction, also called the compound's electromotive potential.



Free energy changes accompany the transfer of electrons between electron donor–acceptor pairs of compounds and are related to the measurable electromotive force of the electron flow. Remember that *in electron transfer, an electron donor reduces the acceptor, and in the process the electron donor becomes oxidized. Consequently, the acceptor, as it is reduced, oxidizes the donor*. The quantity of energy released is directly proportional to the difference in the standard reduction potentials, ΔE_{0'}, between the partners of the redox pair. The free energy of a redox reaction and the ΔE_{0'} of the interacting compounds is related by the expression

$$\Delta G^0' = -nF\Delta E_0'$$

where ΔG^{0'} is the standard free energy change in calories, n is the number of electrons transferred, and F is a constant called the faraday (23,062 cal absolute volt equivalent).

An example of a reduction-oxidation reaction that occurs within the electron transport system is the transfer of hydrogen atoms and electrons from NADH through the flavin mononucleotide (FMN)-linked enzyme NADH dehydrogenase to oxidized coenzyme Q (CoQ). The half-reactions and E_{0'} values for each of these reactions follow:



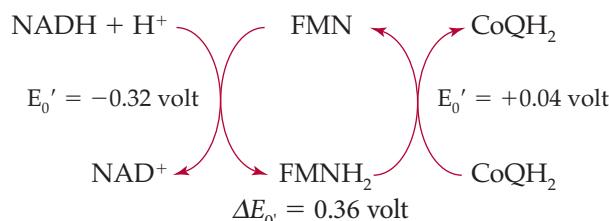
$$E_0' = -0.32 \text{ volt}$$



$$E_0' = +0.04 \text{ volt}$$

Because the NAD⁺ system has a relatively more negative E_{0'} value than the CoQ system, NAD⁺ has a greater reducing potential than the CoQ system, because electrons tend to flow toward the system with the more positive E_{0'}. The reduction of CoQ by NADH therefore is predictable,

and the coupled reaction, linked by the FMN of NADH dehydrogenase, can be written as follows:



SUMMARY

This brief walk through the cell—beginning with its outer surface, the plasma membrane, and moving into its innermost part, where the nucleus is located—provides a view of how this living entity functions. Characteristics of the cell that seem particularly notable are these:

- The flexibility of the plasma membrane in adjusting or reacting to its environment while protecting the rest of the cell as it monitors what may pass into or out of the cell. Prominent in the membrane's reaction to its environment are the receptor proteins, which are synthesized on the rough endoplasmic reticulum and moved through the Golgi apparatus to their intended site on the plasma membrane.
- The communication among the various components of the cell made possible through the cytoplasm, with its microtrabecular network, and also through the endoplasmic reticulum and Golgi apparatus. The networking is such that communications flow not only among components within the cell but also between the nucleus and the plasma membrane.
- The efficient division of labor among the cell components (organelles). Each component has its own specific functions to perform, with little overlap. Furthermore, much evidence is accumulating to support the concept of an “assembly line” not only in oxidative phosphorylation on the inner membrane of the mitochondrion but also in almost all operations, wherever they occur.
- The superb management exercised by the nucleus to ensure that all the proteins needed for a smooth operation are synthesized. Proteins needed as recognition

Inserting this value for $\Delta E_0'$ into the energy equation gives

$$\Delta G^0' = -2(23,062)(0.36) = -16,604 \text{ cal/mol}$$

The amount of energy liberated from this single reduction-oxidation reaction within the electron transport chain therefore is more than enough to phosphorylate ADP to ATP, which, as you'll recall, requires approximately 7,300 cal/mol (35.7 kJ).

markers, receptors, transport vehicles, and catalysts are available as needed.

- The fact that, like all living things, cells must die a natural death. This programmed process is called apoptosis, a particularly attractive focus of current research.

Despite the efficiency of the cell, it is still not a totally self-sufficient unit. Its continued operation is contingent on receiving appropriate and sufficient nutrients. Nutrients needed include not only those that can be used to produce immediate metabolic energy (ATP) or for storage as chemical energy. Most of this energy is needed to maintain normal body temperature, but much of it is also conserved in the form of high-energy phosphate bonds, principally ATP. The ATP can, in turn, activate various substrates by phosphorylation to higher energy levels from which they can undergo metabolism by specific enzymes. The exothermic hydrolysis of the ATP phosphate is sufficient to drive the endothermic phosphorylation, thereby completing the energy transfer from nutrient to metabolite. The oxidative pathways for the macronutrients (carbohydrate, fat, protein, and alcohol) provide a continuous flow of energy for maintaining heat and replenishing ATP. The cell also needs nutrients required as building blocks for structural macromolecules. In addition, the cell must have an adequate supply of the so-called regulatory nutrients (i.e., vitamins, minerals, and water).

With a view of the structure of the “typical cell,” the division of labor among its component parts, and the location within the cell where many of the key metabolic reactions necessary to continue life take place, we can now consider how the cell receives its nourishment.

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www.nmsociety.org

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Masters C, Crane D. *The Peroxisome: A Vital Organelle*. Cambridge, England: Cambridge University Press, 1995.

This is a clearly written overview of the multifaceted functions of this important organelle.

Nutritional Genomics: The Foundation for Personalized Nutrition, by Ruth M. DeBusk, Ph.D., R.D.

What Is Nutritional Genomics?

Nutritional genomics focuses on the interaction among genes and environmental factors, specifically bioactive components in food. This emerging discipline represents fundamental concepts that underlie effective nutrition therapy for disease management and disease prevention. Each person's genetic material (deoxyribonucleic acid or DNA) contains the information essential for the development and function of an organism, including the human body. Genes are units of information within DNA that translate into the myriad of proteins that carry out the work of the body's cells. Genes code for the amino acid sequence of each protein. Noncoding sequences of the DNA molecule are also important; they are key elements in controlling how the various genes are expressed into their protein products.

Because DNA serves as the blueprint of information for each cell, processes have evolved to ensure that the DNA molecule is inherited by new cells from their parent cells and from one generation of humans to the next. The term *genetics* refers to the study of how genes are inherited. In keeping with the times during which this discipline developed, genetics focuses on understanding the role of single genes in the overall functioning of the organism and, thus, a gene's role in health and disease. In contrast, the term *genomics* is a newer one. It also encompasses the study of genes and their functions and inheritance. However, it also includes more global and complex phenomena, such as the effect of specific variations in a gene on an organism's function and its adaptation to its environment and also the influence of environmental factors on gene expression. Nutrition is a major factor in this interplay among genes and the environment in which an organism must function. Not surprisingly, gene-diet interactions are an important focus of current research.

Nutritional genomics is the field of study concerned with these complex interactions among genes and environmental factors. There are two major subcategories of nutritional genomics: nutrigenetics and nutrigenomics. **Nutrigenetics** is concerned with the effect of gene variations (also called gene variants) on the organism's functional ability, specifically its ability to digest, absorb, and use food to sustain life. The particular gene variants a person has determine the nutritional requirements for that person. **Nutrigenomics**, in contrast, is concerned with how bioactive components within food affect gene expression and function. The nomenclature for the

field of nutritional genomics is still evolving, and it is common to see "nutrigenomics" used as a shorthand version of "nutritional genomics." However, keeping the concepts separate can be helpful when sorting out the underlying mechanisms involved. A number of reviews provide an excellent overview of the breadth and depth of nutritional genomics [1–14].

Pharmacogenomics as a Model

The significant advances in genetic technology that resulted from the Human Genome Project have spurred a genomics revolution in health care. Like nutritional genomics, large-scale genomic technologies have enabled pharmacogenomics to move beyond its traditional roots to begin to individualize care.

Pharmacogenomics, the study of how genes and drugs interact, is one of the earliest manifestations of the genomics revolution's practical applications. Physicians have long observed that the same drug at the standard dosage elicits different results in different people. Some will not benefit from the drug, others will be helped in the ways intended, and still others will have serious adverse reactions. Until the integration of genomics into medicine, the approach was to try different dosages and different drugs until an appropriate combination was found. With the advances in genomics research and technology, physicians can now analyze a patient's key drug-metabolizing enzymes and predict which drugs and dosage will be effective for that person.

Nutritional genomics is often likened to pharmacogenomics and predicted to follow the path of pharmacogenomics in terms of clinical applicability. In principle, the two fields are much alike. Genes and the proteins they encode underlie the ultimate physiological outcomes, and variations in the genes affect how well the proteins carry out their functions. However, important differences will cause nutritional genomics to lag considerably behind pharmacogenomics in terms of being integrated into practice.

Pharmacogenomics is able to draw on decades of pharmaceutical research into drug metabolism and the genes involved. In contrast, nutrition research is in its infancy. Gene-diet associations are just now being identified and studied. Additionally, pharmacogenomics involves a single, highly purified compound, administered in a defined chemical form and known amount. Food, in contrast, is composed of a myriad of compounds in highly varying amounts. However, these limitations do not diminish the importance of nutritional genomics. They affect only how quickly

nutritional genomics will achieve widespread clinical integration.

The role of genes in physiological function is fundamental and, therefore, must be integrated into both managing and preventing disease if therapies are to be effective and people are to reach their optimal health potential and, more broadly, their full genetic potential. A recent review by Ghosh and colleagues addresses the synergies and differences between pharmacogenomics and nutritional genomics [15].

Mechanisms Underlying Nutritional Genomics

Nutrigenetics is the more familiar of the two subtypes of nutritional genomics. At one end of the spectrum are the highly penetrant single gene disorders that give rise to inborn errors of metabolism. In this instance a single gene contains a mutation that affects the function of the protein encoded by that gene in a major way. Classic examples include disorders of amino acid metabolism, such as phenylketonuria and maple syrup urine disease; of carbohydrate metabolism, such as galactosemia and hereditary fructose intolerance; and of lipid metabolism, such as familial hypercholesterolemia and medium-chain acyl-CoA dehydrogenase deficiency. Nutrition professionals have long made an important contribution to caring for people with these disorders. For a recent review for nutritional aspects of inborn errors of metabolism see [16].

More recently less penetrant, more subtle variations have been identified that also affect the gene-encoded protein's function. However, such variations do not in themselves cause disease. Instead, they alter a person's susceptibility for developing a disease. Depending on the specific gene variant, the person's likelihood of developing a disorder may be increased or reduced. These genes are the primary focus of nutritional genomics, because they are common within the global population, they affect dietary recommendations about the types and amounts of food that best fit a person, and practical interventions are possible. These interventions can potentially improve the health potential of individual people and, by extrapolation, the populations in which they live.

For the numerous genes that are influenced by dietary factors, such variations offer the potential to manipulate the diet and thereby modulate genetic outcomes. For example, the 677C>T variation in the gene for 5,10-methylenetetrahydrofolate reductase (MTHFR) results in an altered enzyme that, in the absence of adequate folate, increases the risk for

colon cancer, fetal neural tube defects, and possibly cardiovascular disease. People with this variation must be particularly diligent in maintaining adequate folate intake and, quite possibly, may need more than the standard recommended amount [17]. Ames and colleagues published a seminal paper in 2002 detailing at least 50 enzymes, involved in metabolism, with gene variants that decreased the enzymes' function [18]. People with these variants needed more than the recommended nutrient levels to compensate for the decreased function of these enzymes. On the other hand, people with certain variations in the *APOA1* gene, which encodes the major protein of high-density lipoprotein cholesterol, are relatively immune to saturated fat in the diet. Clearly, knowing a person's genotype is a critical factor when developing dietary recommendations. For discussions of genetic variations and their effects on nutrient requirements, see references [19–21].

Nutrigenomics reflects the complex communication that occurs between the environment and an organism's command center. Even the most primitive bacterium has a mechanism by which it monitors the nutritional sufficiency of its environment and transmits that information to its genetic material. This information triggers increased or decreased gene expression, as appropriate. Consider the *lac* and *trp* operons in bacteria, in which external food molecules cause specific gene expression to be turned on or off, respectively. Humans have similar communication processes by which important environmental information is transferred to the nucleus. There, it influences gene expression, either by direct interaction with DNA or through signal transduction, by which molecular events at the cell membrane are transmitted to the DNA.

The details of the mechanisms by which gene expression is modulated are under investigation. The primary mechanism appears to be transcriptional regulation. Specific DNA sequences in the regulatory region upstream from the coding region of a gene serve as response elements, to which regulatory proteins (called transcription factors) bind. Binding leads to conformational changes in the DNA molecule. These changes either permit or inhibit RNA polymerase from attaching to the promoter region and initiating transcription of the message encoded in the gene. The binding of transcription factors to response elements is influenced by various ligands. For example, transcription of a number of genes involved in lipid metabolism and oxidation requires the coordinated binding of two transcription factors: the retinoic

acid receptor (RXR) and the peroxisome proliferator activator receptor gamma (PPARgamma). These two proteins must form a heterodimer in order to bind to the response element in the regulatory region of these genes. To form the heterodimer, each protein must bind its respective ligand. For RXR, the ligand is a vitamin A derivative; for PPARgamma, the ligand is a polyunsaturated fatty acid, such as an omega-3 fat. Thus, food components are integral in communicating the state of the environment to the command center and effecting the appropriate response. Numerous transcription factors and their response elements and effector ligands are being studied. A discussion of dietary fatty acid interaction with various lipid-sensitive transcription factors, with particular emphasis on dyslipidemia and the metabolic syndrome is provided [22,23].

Such an understanding of the underlying mechanisms, coupled to information about a person's genotype, forms the basis for developing targeted nutritional interventions. For example, knowing that omega-3 fats down-regulate key genes involved in chronic inflammation provides a logical rationale for developing diet and lifestyle recommendations for people whose genotype puts them at increased risk for developing chronic inflammation [24–26].

Nutritional Genomics and Lipid Metabolism

From a health perspective, the major concerns regarding genes and lipid metabolism center around susceptibility to vascular disease [27, 28]. Genes involved with cholesterol homeostasis offer examples of how genetic variations affect lipid metabolism and, thereby, disease risk. They also present opportunities for nutritional genomics to guide diet and lifestyle choices that can minimize one's risks. Examples of such diet-gene interactions include the genes *APOE*, *APOA1*, and *CETP*.

The *APOE* gene encodes a protein that facilitates the interaction among triglyceride-rich chylomicrons, intermediate-density lipoprotein particles, and their respective receptors. This gene has three common variants (alleles): E2, E3, and E4. E3 is the most common form. Six possible genotypes can occur: E2/E2, E2/E3, E3/E3, E3/E4, and E4/E4. Corella and Ordovas review the numerous studies that have investigated the diet-gene interaction for *APOE* variants [29]. Dietary response varies with both the allele present and the number of copies. In general, people with at least one E4 allele have the highest basal levels of various lipids and show the greatest lipid-lowering response to a low-fat diet. Those

with at least one copy of the E2 allele have the lowest basal lipid levels and are helped least by a low-fat diet.

Taking into account which *APOE* alleles a person has is helpful in developing diet and lifestyle interventions for improving serum lipid levels. Those with one or more E2 alleles tend to have the lowest serum total cholesterol, low-density lipoprotein-cholesterol (LDL-C), and apoB levels and the highest triglyceride levels of the six possible genotypes. Such people are the least responsive to a low-fat diet but appear to respond well to oat bran and other soluble fibers [30]. They also can lower serum triglyceride level with fish oil supplementation [31]. Endurance exercise is particularly effective in increasing HDL-cholesterol levels [32].

In contrast, those with one or more E4 alleles have the highest serum total cholesterol, LDL-C, and apoB levels, the lowest HDL-C levels, and have elevated fasting and postprandial triglyceride levels [29]. They respond best to a low-fat diet but are the least responsive to soluble fiber for lowering serum lipids or to exercise for increasing HDL levels. Fish oil supplementation in these people increases total cholesterol and reduces HDL [31]. Key lifestyle choices to be aware of with this genotype are alcohol and smoking. Alcohol increases LDL-C levels and does not beneficially increase HDL-C levels [33,34], and smoking increases both LDL-C levels and carotid artery intima-media thickening [35,36]. Whether a person has the E2 allele or the E4 allele appears to make a difference in the diet and lifestyle recommendations that would be appropriate for improving vascular health.

The *APOA1* gene codes for apolipoprotein A-1, the primary protein in high-density lipoprotein (HDL). The -75G>A variant has a single nucleotide change in which the guanine component has been replaced with an adenine at position 75 within the regulatory region of the *APOA1* gene. This change affects HDL levels in response to low-fat diets [37]. A common practice in treating dyslipidemia is to reduce the saturated fat content of the diet and increase the polyunsaturated fat content. Typically, HDL levels fall in women with the more common G allele as the polyunsaturated content of the diet increases, an effect counter to the desired one. These women would benefit from a fat-modified diet that keeps amounts of both saturated and polyunsaturated fat low and increases amounts of monounsaturated fat. Women with the A allele, however, respond differently. Increasing polyunsaturated dietary fat leads to increased HDL levels, and the effect is "dose-dependent," meaning that the increase is more dramatic in the presence of two A alleles than it is with just one. For these women, a diet low in saturated fat,

moderate in polyunsaturated fat (8% or greater of total calories), and supplying the rest in monounsaturated fat has the greatest benefit in raising HDL levels. Clearly, whether a person has the -75G>A *APOA1* variant, and how many copies are present, will affect any therapeutic intervention developed to correct dyslipidemia.

Another gene that affects HDL levels is *CETP*, which codes for the cholesteryl ester transfer protein that transfers cholesteryl esters from HDL to other lipoproteins. This protein is also called the "lipid transfer protein." People with two copies of a common allele at position 279 of this gene tend to have low HDL levels and elevated levels of LDL and VLDL. A variation (279G>A) that decreases plasma levels of *CETP* is associated with increased HDL levels, decreased LDL and VLDL levels, and a lower risk of cardiovascular disease than the more common (GG) form [38]. These people are responsive to alcohol and further increase their HDL levels with regular moderate intake [39].

Once gene variants relating to dietary manipulation of lipid metabolism have been detected, diet and lifestyle interventions can be factored in when developing therapeutic interventions. In addition to manipulating the macronutrient content of the diet, adding many functional foods and dietary supplements can help in achieving desired outcomes. The continued discovery of how bioactive components within food affect gene expression is leading to an increasingly targeted use of food and isolated food components to achieve desired outcomes. Similarly, understanding how lifestyle choices, such as exercise and exposure to tobacco smoke or other environmental toxins, interact with specific gene variants creates yet another set of tools for promoting health.

Opportunities for Nutrition Professionals

The opportunities for nutrition professionals with competency in nutritional genomics are expanding and promise to be an integral part of future nutrition practice in all its manifestations. From research opportunities to food science opportunities, nutritional genomics will promote research into genes and their interactions with dietary components; genetic testing technologies; the development of gene-based nutritional interventions; the isolation, characterization, and possible selling of bioactive components from food; and the development of functional foods targeted to particular genotypes. Sales and marketing research related to functional foods, dietary supplements, and genetic technologies will present additional opportunities. Education will be an

ongoing need at all levels, from health care professionals to food and nutrition students to the lay public. The opportunities appear quite varied. See DeBusk et al. [40] for a discussion of nutrition practice in the age of nutritional genomics.

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2

The Digestive System: Mechanism for Nourishing the Body

The Structures of the Digestive Tract and the Digestive Process

The Oral Cavity
The Esophagus
The Stomach
The Small Intestine
The Accessory Organs
The Digestive and Absorptive Processes
The Colon or Large Intestine

Coordination and Regulation of the Digestive Process

Neural Regulation
Regulatory Peptides

Summary

PERSPECTIVE

An Overview of Selected Digestive System Disorders with Implications for Nourishing the Body

Nutrition is the science of nourishment. Ingestion of foods and beverages provides the body with at least one, if not more, of the nutrients needed to nourish the body. The body needs six classes of nutrients: carbohydrate, lipid, protein, vitamins, minerals, and water. For the body to use the carbohydrate, lipid, protein, and some vitamins and minerals found in foods, the food must be digested first. In other words, the food first must be broken down mechanically and chemically. This process of digestion occurs in the digestive tract and, once complete, yields nutrients ready for absorption and use by the body.

The Structures of the Digestive Tract and the Digestive Process

The digestive tract, approximately 16 ft in length, includes organs that comprise the alimentary canal (also called the gastrointestinal tract or gut) as well as certain accessory organs. The main structures of the digestive tract include the oral cavity, esophagus, and stomach (collectively referred to as the upper digestive tract), and the small and large intestines (called the lower digestive tract). The accessory organs include the pancreas, liver, and gallbladder. The accessory organs provide or store secretions that ultimately are delivered to the lumen of the digestive tract and aid in the digestive and absorptive processes. Figure 2.1 illustrates the digestive tract and accessory organs. Figure 2.2 provides a cross-sectional view of the gastrointestinal tract that shows the lumen (interior passageway) and the four main tunics, or layers, of the gastrointestinal tract:

- the mucosa
- the submucosa
- the muscularis externa
- the serosa, or adventitia

Some of these layers contain sublayers. The mucosa, the innermost layer, is made of three sublayers: the epithelium or epithelial lining, the lamina propria, and the muscularis mucosa. The mucosal epithelium, which lines the lumen of the gastrointestinal tract, is the surface that is in contact with nutrients in the food we eat. Exocrine and endocrine cells also are found among the epithelial cells of the mucosa. The exocrine cells secrete a variety of substances, such as enzymes and juices, into the lumen of the gastrointestinal tract, and the endocrine cells secrete various hormones into the blood. The lamina propria lies below the epithelium

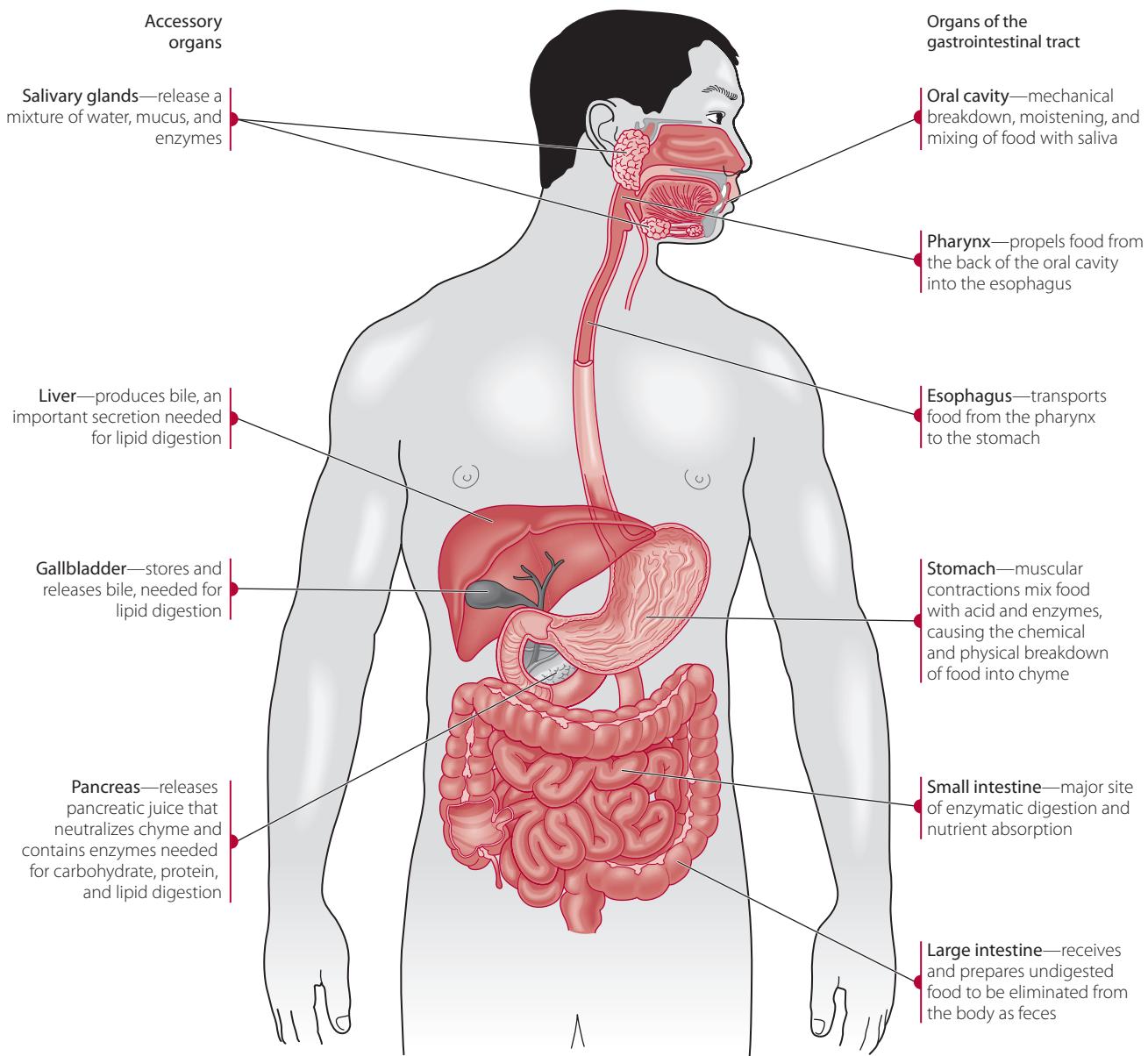


Figure 2.1 The digestive tract and its accessory organs.

and consists of connective tissue and small blood and lymphatic vessels. Lymphoid tissue also is found within the lamina propria. This lymphoid tissue contains a number of white blood cells, especially macrophages and lymphocytes, which provide protection against ingested microorganisms. The third sublayer of the mucosa, the muscularis mucosa, consists of a thin layer of smooth muscle.

Next to the mucosa is the submucosa. The submucosa, the second tunic or layer, is made up of connective tissue and more lymphoid tissue and contains a network of nerves called the submucosal plexus, or plexus of Meissner. This plexus controls, in part, secretions from the mucosal glands and helps regulate mucosal movements and blood flow. The lymphoid tissue in the submucosa is similar to that found in the mucosa and protects the body against

foreign substances. The submucosa binds the first mucosal layer of the gastrointestinal tract to the muscularis externa, or third layer of the gastrointestinal tract.

The muscularis externa contains both circular and longitudinal smooth muscle, important for peristalsis, as well as the myenteric plexus, or plexus of Auerbach. This plexus controls the frequency and strength of contractions of the muscularis to affect gastrointestinal motility.

The outermost layer, the serosa or adventitia, consists of connective tissue and the visceral peritoneum. The peritoneum is a membrane that surrounds the organs of the abdominal and pelvic cavities. In the abdominal cavity, the visceral peritoneum surrounds the stomach and intestine, and the parietal peritoneum lines the cavity walls. The arrangement creates a double-layered membrane

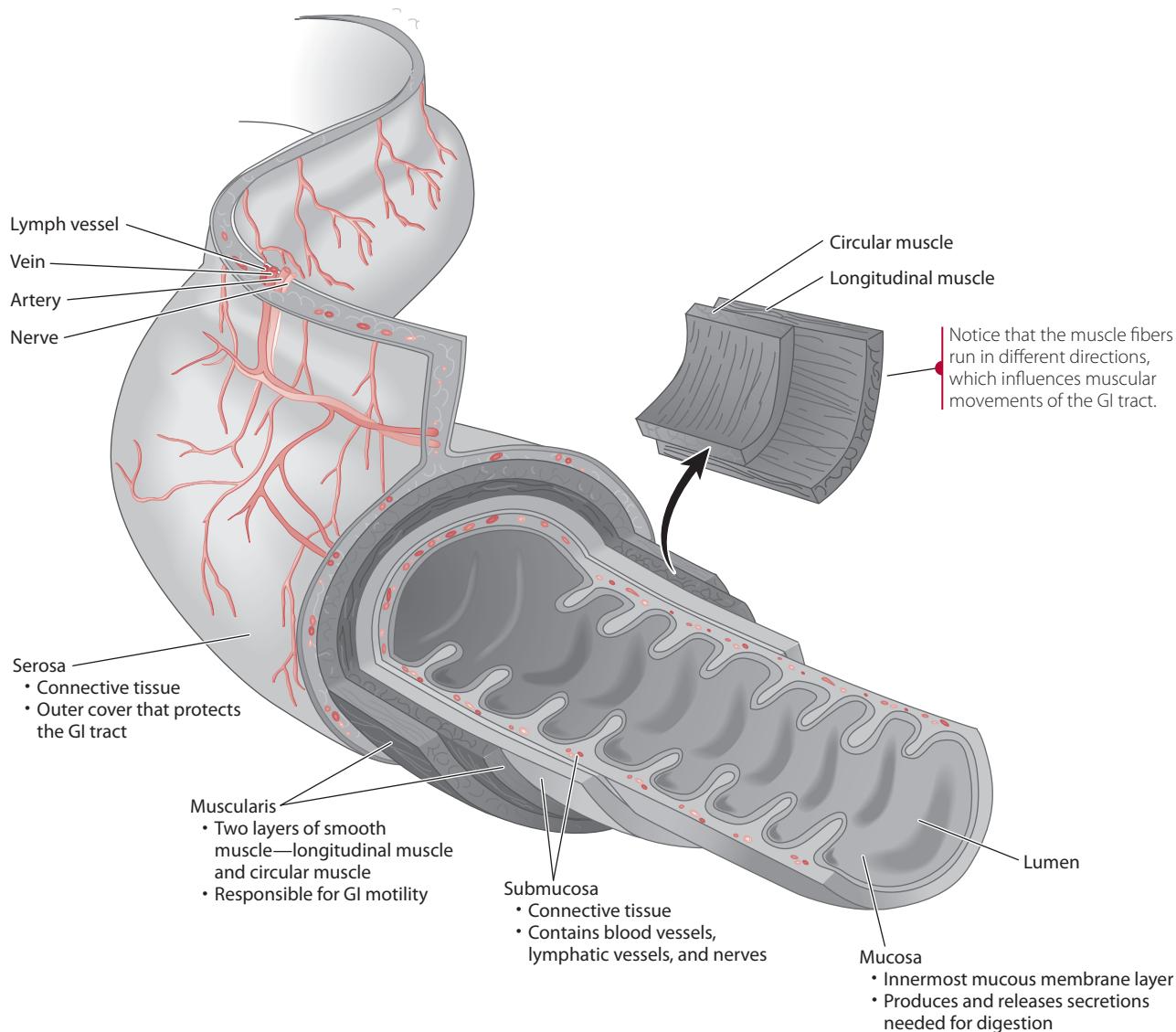


Figure 2.2 The sublayers of the small intestine.

within the abdominal cavity. These membranes are somewhat permeable and highly vascularized. Between the two membranes is the peritoneal cavity. The selective permeability and the rich blood supply of peritoneal membranes allow the peritoneal cavity to be used in the treatment of renal failure, a process called dialysis.

The digestive process begins in the oral cavity and proceeds sequentially through the esophagus, stomach, small intestine, and finally into the colon (large intestine). The next subsections of this chapter describe the structures and digestive processes that occur in each of these parts of the digestive tract. Other sections include information on the structures and roles of the pancreas, liver, and gallbladder and the roles of a variety of enzymes. Table 2.1 provides an overview of some of the enzymes and **zymogens** (pro-enzymes or inactive enzymes, which must be chemically altered to function as an enzyme) that participate in digesting the nutrients in foods.

THE ORAL CAVITY

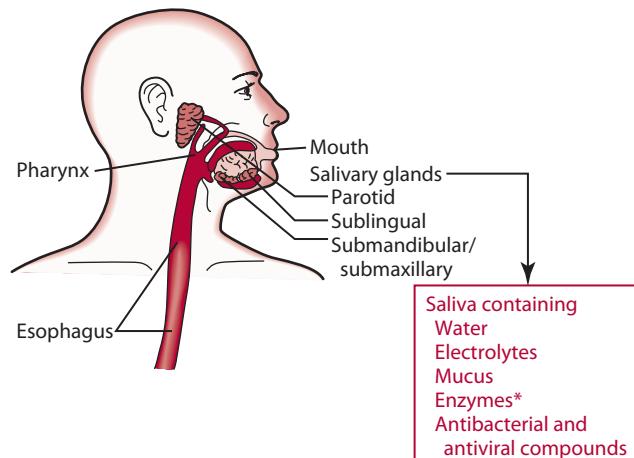
The mouth and pharynx (or throat) constitute the oral cavity and provide the entryway to the digestive tract. On entering the mouth, food is chewed by the actions of the teeth and jaw muscles and is made ready for swallowing by mixing with secretions (saliva) released from the salivary glands. Three pairs of small, bilateral saliva secreting salivary glands—the parotid, the submandibular, and the sublingual—are located throughout the lining of the oral cavity, along the jaw from the base of the ear to the chin (Figure 2.3). These glands are affected by the actions of the parasympathetic and sympathetic nervous systems. Secretions (about 1 L/day) from these glands constitute saliva. Specifically, the parotid glands secrete water, electrolytes (sodium, potassium, chloride), and enzymes. The submandibular and sublingual glands secrete water, electrolytes, enzymes, and mucus. Saliva is primarily (99.5%) water, which helps dissolve foods. The principal enzyme

of saliva is α amylase (also called ptyalin) (Table 2.1). This enzyme hydrolyzes internal α 1-4 bonds within starch. A second digestive enzyme, lingual lipase, is produced by lingual serous glands on the tongue and in the back of the mouth. This enzyme hydrolyzes dietary triacylglycerols (triglycerides) in the stomach, but its activity both diminishes with age and is limited by the coalescing of the fats within the stomach. Activity of lingual lipase in infants against triglycerides in milk improves digestion of dietary fats. Mucus secretions found in saliva contain **glycoproteins** (compounds consisting of both carbohydrates and proteins). Mucus lubricates food and coats and protects the oral mucosa. Antibacterial and antiviral compounds, one example being the antibody IgA (immunoglobulin A), along with trace amounts of organic substances (such as urea) and other solutes (i.e., phosphates, bicarbonate), are also found in saliva.

THE ESOPHAGUS

From the mouth, food, now mixed with saliva and called a bolus, is passed through the pharynx into the esophagus. The esophagus is about 10 inches long (Figure 2.1). The

passage of the bolus of food from the oral cavity into the esophagus constitutes swallowing. Swallowing, which can be divided into several stages—voluntary, pharyngeal, and esophageal—is a reflex response initiated by a voluntary



*Main enzyme in saliva is salivary amylase, which hydrolyzes α 1-4 bonds in starch.

Figure 2.3 Secretions of the oral cavity.

Table 2.1 Digestive Enzymes and Their Actions

Enzyme or Zymogen/Enzyme	Site of Secretion	Preferred Substrate(s)	Primary Site of Action
Salivary α amylase	Mouth	α 1-4 bonds in starch, dextrans	Mouth
Lingual lipase	Mouth	Triacylglycerol	Stomach, small intestine
Pepsinogen/pepsin	Stomach	Carboxyl end of phe, tyr, trp, met, leu, glu, asp	Stomach
Gastric lipase	Stomach	Triacylglycerol (mostly medium chain)	Stomach
Trypsinogen/trypsin	Pancreas	Carboxyl end of lys, arg	Small intestine
Chymotrypsinogen/chymotrypsin	Pancreas	Carboxyl end of phe, tyr, trp, met, asn, his	Small intestine
Procarboxypeptidase/carboxypeptidase A	Pancreas	C-terminal neutral amino acids	Small intestine
carboxypeptidase B	Pancreas	C-terminal basic amino acids	Small intestine
Proelastase/elastase	Pancreas	Fibrous proteins	Small intestine
Collagenase	Pancreas	Collagen	Small intestine
Ribonuclease	Pancreas	Ribonucleic acids	Small intestine
Deoxyribonuclease	Pancreas	Deoxyribonucleic acids	Small intestine
Pancreatic α amylase	Pancreas	α 1-4 bonds, in starch, maltotriose	Small intestine
Pancreatic lipase and colipase	Pancreas	Triacylglycerol	Small intestine
Phospholipase	Pancreas	Lecithin and other phospholipids	Small intestine
Cholesterol esterase	Pancreas	Cholesterol esters	Small intestine
Retinyl ester hydrolase	Pancreas	Retinyl esters	Small intestine
Amino peptidases	Small intestine	N-terminal amino acids	Small intestine
Dipeptidases	Small intestine	Dipeptides	Small intestine
Nucleotidase	Small intestine	Nucleotides	Small intestine
Nucleosidase	Small intestine	Nucleosides	Small intestine
Alkaline phosphatase	Small intestine	Organic phosphates	Small intestine
Monoglyceride lipase	Small intestine	Monoglycerides	Small intestine
Alpha dextrinase or isomaltase	Small intestine	α 1-6 bonds in dextrans, oligosaccharides	Small intestine
Glucoamylase, glucosidase, and sucrase	Small intestine	α 1-4 bonds in maltose, maltotriose	Small intestine
Trehalase	Small intestine	Trehalose	Small intestine
Disaccharidases	Small intestine		Small intestine
Sucrase		Sucrose	
Maltase		Maltose	
Lactase		Lactose	

action and regulated by the swallowing center in the medulla of the brain. To swallow food, the esophageal sphincter relaxes, allowing the esophagus to open. Food then passes into the esophagus. Simultaneously, the larynx (part of the respiratory tract) moves upward, inducing the epiglottis to shift over the glottis. The closure of the glottis is important in keeping food from entering the trachea, which leads to the lungs. Once food is in the esophagus, the larynx shifts downward to allow the glottis to reopen.

When the bolus of food moves into and down the esophagus, both the striated (voluntary) muscle of the upper portion of the esophagus and the smooth (involuntary) muscle of the distal portion are stimulated by cholinergic (parasympathetic) nerves. The result is **peristalsis**, a progressive wavelike motion that moves the bolus through the esophagus into the stomach. The process usually takes less than 10 seconds.

At the lower (distal) end of the esophagus, just above the juncture with the stomach, lies the gastroesophageal sphincter, also called the lower esophageal sphincter (Figure 2.4). The presence of this sphincter may be a misnomer, because no consensus exists about whether this particular muscle area is sufficiently hypertrophied to constitute a true sphincter. Several sphincters or valves, which are circular muscles, are located throughout the digestive tract; these sphincters allow food to pass from one section of the gastrointestinal tract to another. On swallowing, the gastroesophageal sphincter pressure drops. This drop in gastroesophageal sphincter pressure relaxes the sphincter so that food may pass from the esophagus into the stomach.

Multiple mechanisms, including neural and hormonal, regulate gastroesophageal sphincter pressure. The musculature of the gastroesophageal sphincter has a tonic pressure

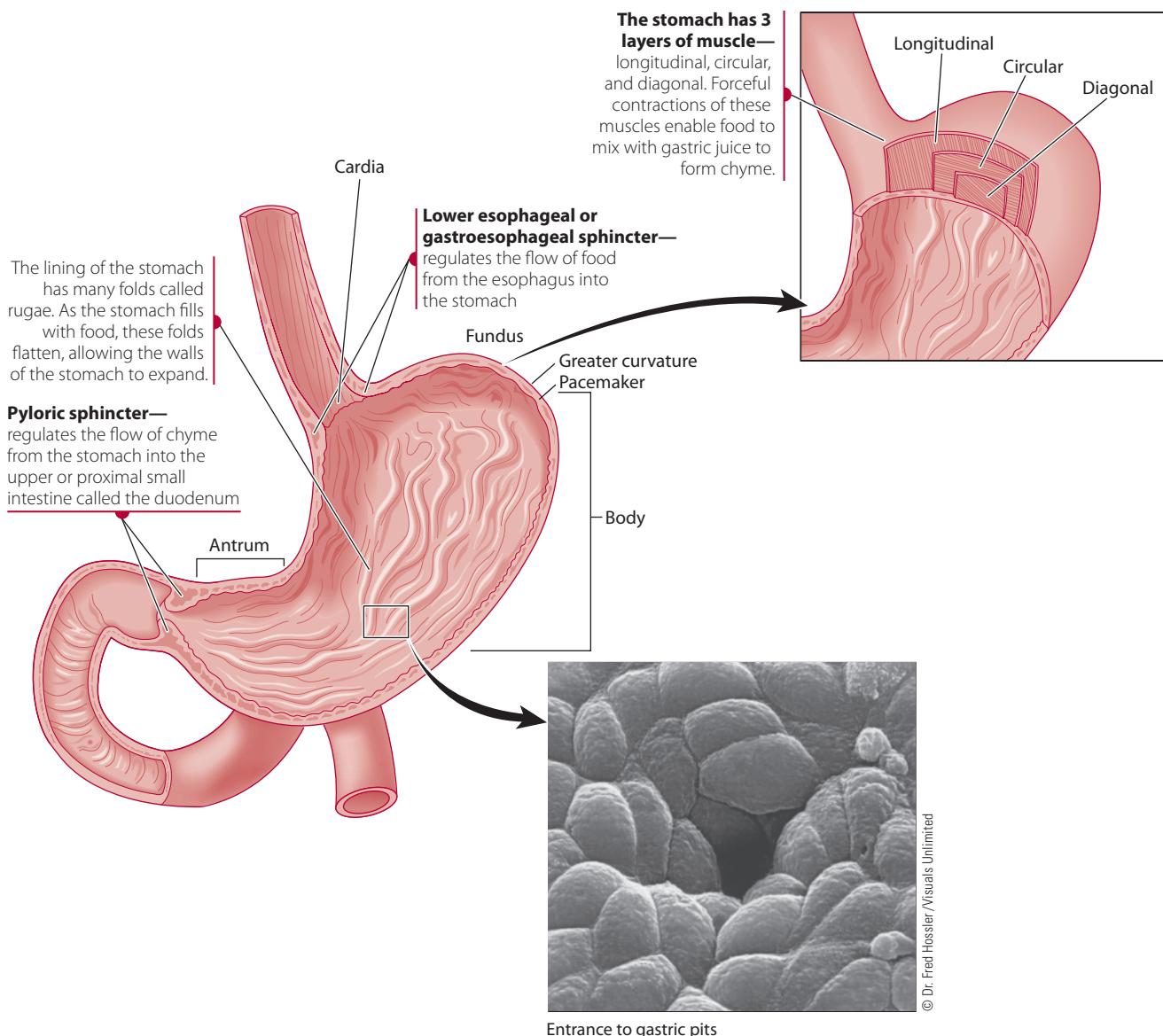


Figure 2.4 Structure of the stomach.

that is normally higher than the intragastric pressure (the pressure within the stomach). This high tonic pressure at the gastroesophageal sphincter keeps the sphincter closed. Keeping this sphincter closed is important, because it prevents gastroesophageal reflux: the movement of substances from the stomach back into the esophagus.

Selected Diseases and Conditions of the Esophagus

A person experiencing gastroesophageal reflux feels a burning sensation in the midchest, a condition referred to as heartburn. Gastric acid, when refluxed from the stomach and present in the esophagus, is an irritant to the esophageal mucosa. Repeated exposure of the esophageal mucosa to this gastric acid can irritate the esophagus and lead to esophagitis, or inflammation of the esophagus. Foods and food-related substances can indirectly affect gastroesophageal sphincter pressure and cause reflux. Smoking, chocolate, high-fat foods, alcohol, and carminatives such as peppermint and spearmint, for example, promote relaxation of the gastroesophageal sphincter and increase the likelihood of acid reflux into the esophagus. Gastroesophageal reflux disease, reflux esophagitis, and treatments for these conditions are described in the Perspective at the end of this chapter.

THE STOMACH

Once the bolus of food has passed through the gastroesophageal sphincter, it enters the stomach, a J-shaped organ located on the left side of the abdomen under the diaphragm. The stomach extends from the gastroesophageal sphincter to the duodenum, the upper or proximal section of the small intestine. The stomach contains four main regions (shown in Figure 2.4):

- The cardia region lies below the gastroesophageal sphincter and receives the swallowed food from the esophagus.
- The fundus lies adjacent or lateral to and above the cardia.
- The large central region of the stomach is called the body. The body of the stomach serves primarily as the reservoir for swallowed food and is the main production site for gastric juice.
- The antrum or distal pyloric portion of the stomach consists of the lower or distal one-third of the stomach.

The antrum grinds and mixes food with the gastric juices, thus forming a semiliquid **chyme** (partially digested food existing as a thick semiliquid mass). The antrum also provides strong peristalsis for gastric emptying through the pyloric sphincter into the duodenum. The pyloric sphincter is found at the juncture of the stomach and duodenum.

The stomach begins mixing the food with gastric juices and enzymes using circular, longitudinal, and oblique smooth muscles of the stomach. It holds the partially digested chyme before releasing it in small quantities, at regular intervals, into the duodenum. The volume of the stomach when empty (resting) is about 50 mL (~2 oz), but on being filled it can expand to accommodate from 1 L to approximately 1.5 L (~37–52 oz) or more. When the stomach is empty, folds (called rugae, see Figure 2.4) present in all but the antrum section of the stomach are visible; however, when the stomach is full, the rugae disappear.

The digestive process is facilitated by gastric juices, which are produced in significant quantities by glands in the body of the stomach. The stomach is lined with epithelial cells that contain millions of gastric glands. Gastric juice is produced by three functionally different gastric glands, found within the gastric mucosa and submucosa of the stomach:

- the cardiac glands, found in a narrow rim at the juncture of the esophagus and the stomach
- the oxytic glands, found in the body of the stomach
- the pyloric glands, located primarily in the antrum

Several cell types, which secrete different substances, may be found within a gastric gland, as shown in Figure 2.5. For example, some of the cells found in a gastric oxytic gland include:

- neck (mucus) cells, located close to the surface mucosa, which secrete bicarbonate and mucus
- parietal (oxytic) cells, which secrete hydrochloric acid and intrinsic factor
- chief (peptic or zymogenic) cells, which secrete pepsinogens
- enteroendocrine cells, which secrete a variety of hormones

Unlike the oxytic glands, the cardiac glands contain no parietal cells. The pyloric glands contain mucus and parietal cells, as well as enteroendocrine cells called G-cells.

The main constituents of gastric juice produced by the different cells of the gastric glands include water, electrolytes, hydrochloric acid, enzymes, mucus, and intrinsic factor. The next section describes some of the main constituents—hydrochloric acid, enzymes, and mucus—of gastric juice.

Gastric juice contains an abundance of hydrochloric acid secreted from gastric parietal cells (Figure 2.6). Parietal cells contain both a potassium chloride transport system and a hydrogen (proton) potassium ATPase exchange system. The potassium chloride system transports both ions into the gastric lumen. The hydrogen potassium ATPase system (H^+, K^+ -ATPase), also referred to as a proton pump, allows the exchange of two potassium ions for two hydrogens

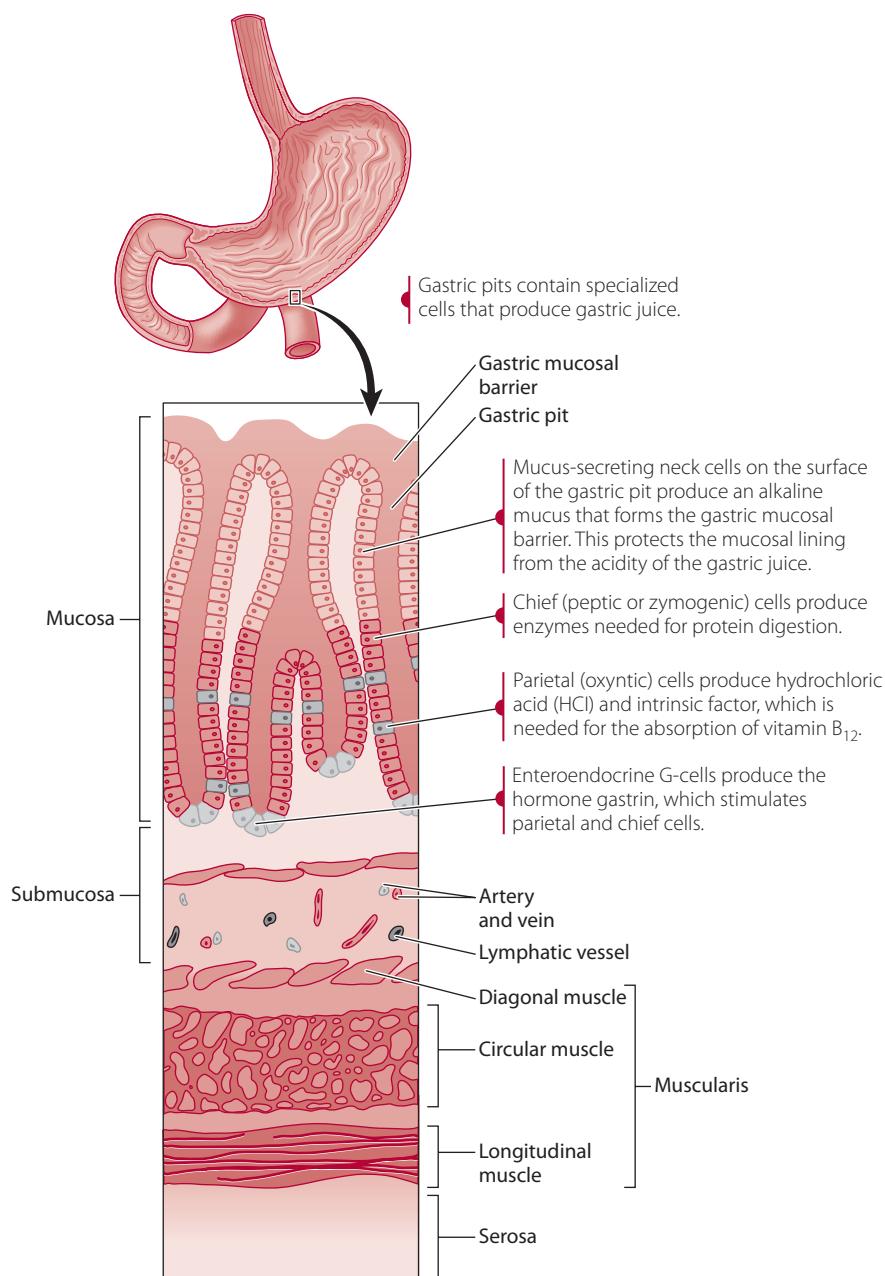


Figure 2.5 A gastric gland and its secretions in the body of the stomach.

(protons) with each ATP molecule hydrolyzed. Some diffusion of chloride into and out of the parietal cell and some diffusion of potassium into gastric juice also have been proposed. Nonetheless, the net effect is that hydrogen and chloride or hydrochloric acid are secreted into the gastric lumen as part of gastric juice. The high concentration of hydrochloric acid in the gastric juice is responsible for its low pH, about 2. The pH value is the negative logarithm of the hydrogen ion concentration. The lower the pH is, the more acidic the solution is. Figure 2.7 shows the approximate pH values of body fluids and, for comparison, some other compounds and beverages. Notice that the pH of orange juice (and typically of all fruit juices) is higher than that of gastric juice. Thus, drinking such juices cannot lower

the gastric pH. Hydrochloric acid has several functions in gastric juice, including:

- converting or activating the zymogen pepsinogen to form pepsin
- denaturing proteins, which results in the destruction of the tertiary and secondary protein structure and thereby opens interior bonds to the proteolytic effect of pepsin
- releasing various nutrients from organic complexes
- acting as bactericide agent, killing many bacteria ingested along with food

Three enzymes (Table 2.1) are found in gastric juice. The main enzyme, pepsin, is made by the chief cells and

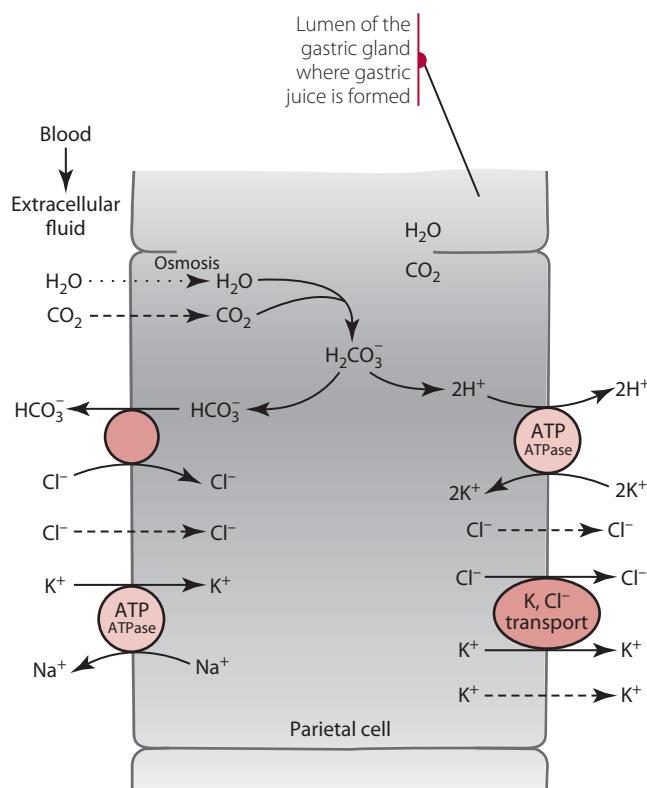


Figure 2.6 A proposed mechanism by which hydrochloric acid (HCl) is secreted into the stomach by parietal cells. Dashed line indicates diffusion. An empty circle indicates non-energy dependent transport.

functions as the principal proteolytic enzyme in the stomach. Pepsin is derived from either of two pepsinogens, I or II. Pepsinogen I is found primarily in the body of the stomach, where most hydrochloric acid is secreted. Pepsinogen II is found in both the body and the antrum of the stomach. The distinction between the two groups of pepsinogens has no known implications for digestion; however, higher concentrations of pepsinogen I correlate positively with acid secretion and have been associated with an increased incidence of peptic ulcers. Pepsinogens are secreted in granules into the gastric lumen from chief cells when they are stimulated by acetylcholine, acid, or both. Pepsinogens can be converted to pepsin, an active enzyme, in an acid environment ($\text{pH} < 5$) or in the presence of previously formed pepsin.

Acid or pepsin



Pepsin functions as a protease, an enzyme that hydrolyzes proteins. Specifically, pepsin is an **endopeptidase**, meaning that it hydrolyzes interior peptide bonds within proteins. Optimal pepsin activity occurs at about $\text{pH } 3.5$. Another enzyme present in the gastric juice is α amylase, which originates from the salivary glands in the mouth. This enzyme, which hydrolyzes starch, retains some activity in the stomach until it is inactivated by the low pH of gastric juice. The

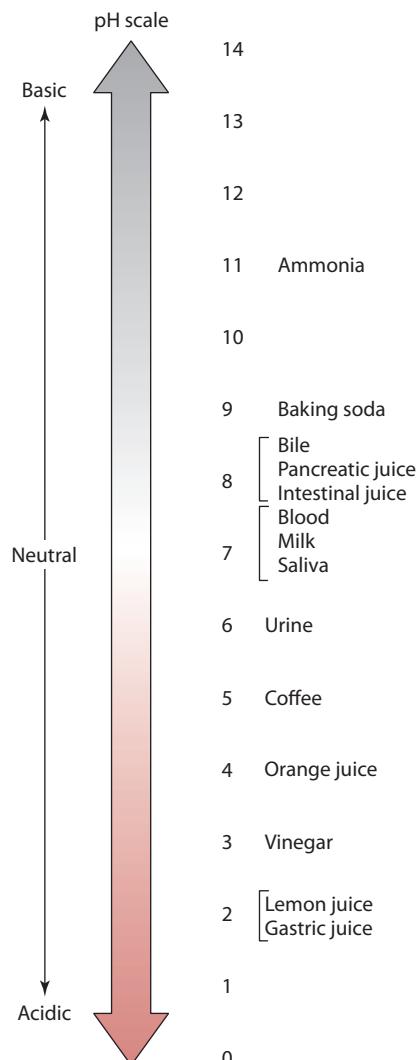


Figure 2.7 Approximate pHs of selected body fluids, compounds, and beverages.

third enzyme found in gastric juice is gastric lipase, which is made by chief cells. Gastric lipase hydrolyzes primarily short- and medium-chain triacylglycerols and is thought to be responsible for up to about 20% of lipid digestion in humans. Additional information about pepsin and amylase can be found in Chapters 6 and 3, respectively. Gastric lipase is discussed further in Chapter 5.

Mucus, secreted by gastric neck or mucus cells, also is found in gastric juice. Secretion of mucus is stimulated by various prostaglandins and by nitric oxide. Mucus, which consists of a network of glycoproteins (mucin), glycolipids, water, and bicarbonate ions (HCO_3^-), lubricates the ingested gastrointestinal contents and coats and protects the gastric mucosa from mechanical and chemical damage. Mucus forms a layer about 2 mm thick on top of the gastric mucosa. Tight junctions between gastric cells also help prevent H^+ from penetrating into the gastric mucosa and initiating peptic ulcer formation.

Another constituent of gastric juice is intrinsic factor. Intrinsic factor is secreted by parietal cells and is necessary

to absorb vitamin B₁₂. Intrinsic factor is discussed in more detail in Chapter 9, “The Water-Soluble Vitamins.”

In summary, gastric juice contains several important compounds that aid in the digestive process. However, very little chemical digestion of nutrients occurs in the stomach except for the initiation of protein hydrolysis by the protease pepsin and the limited continuation of starch hydrolysis by salivary α amylase. The only absorption that occurs in the stomach is that of water, alcohol, a few fat-soluble drugs such as aspirin, and a few minerals. The hydrochloric acid and intrinsic factor generated in the stomach are important for absorbing nutrients such as iron and especially vitamin B₁₂ respectively. For example, nourishment and survival are possible without the stomach as long as a person receives injections of vitamin B₁₂. Nevertheless, a healthy stomach makes attaining adequate nourishment much easier.

Regulation of Gastric Secretions

Gastric secretions are regulated by multiple mechanisms including various hormones and peptides. Several of these hormones and peptides and their actions are shown in Figure 2.8. Hormones that inhibit gastric secretions include peptide YY, enterogastrone, glucose-dependent insulinotropic peptide (formerly called gastric inhibitory peptide—GIP), and secretin. Somatostatin, synthesized by pancreatic and intestinal cells, acts in a paracrine fashion by entering gastric juice, and inhibits gastric secretions. The release of gastric secretions also is inhibited by the neuropeptides vasoactive intestinal polypeptide (VIP) and substance P, some prostaglandins, and nitric oxide.

In contrast, other hormones and neuropeptides stimulate gastric secretions. Gastrin-releasing peptide (GRP), also called bombesin, is released from enteric nerves and stimulates gastrin and hydrochloric acid release. Gastrin, synthesized primarily by enteroendocrine G-cells in the stomach and proximal small intestine, acts on parietal cells directly to stimulate hydrochloric acid release as well as on chief cells to stimulate pepsinogen release. Gastrin also stimulates gastric motility and the cellular growth of (that is, has trophic action on) the stomach. Gastrin release occurs in response to vagal stimulation, gastric distention, hydrochloric acid in contact with gastric mucosa, as well as gastrin-releasing peptide, epinephrine, and ingestion of specific substances or nutrients such as coffee, alcohol, calcium, amino acids, and peptides. The role of gastrin in acid secretion is especially evident in people with Zollinger-Ellison syndrome. This condition, usually caused by a tumor, is characterized by extremely copious secretion of gastrin into the blood, which produces higher than normal blood concentrations of gastrin (referred to as hypergastrinemia). Hypergastrinemia leads to gastric hypersecretion and the formation of multiple ulcers in the stomach, duodenum, and sometimes even the jejunum.

In addition to being stimulated by gastrin, acid release into the stomach is also stimulated by other means. For example, the vagus nerve releases acetylcholine and stimulates the release of histamine. Both acetylcholine and histamine stimulate acid secretion. Moreover, gastrin also stimulates histamine release. Thus, direct mediators or

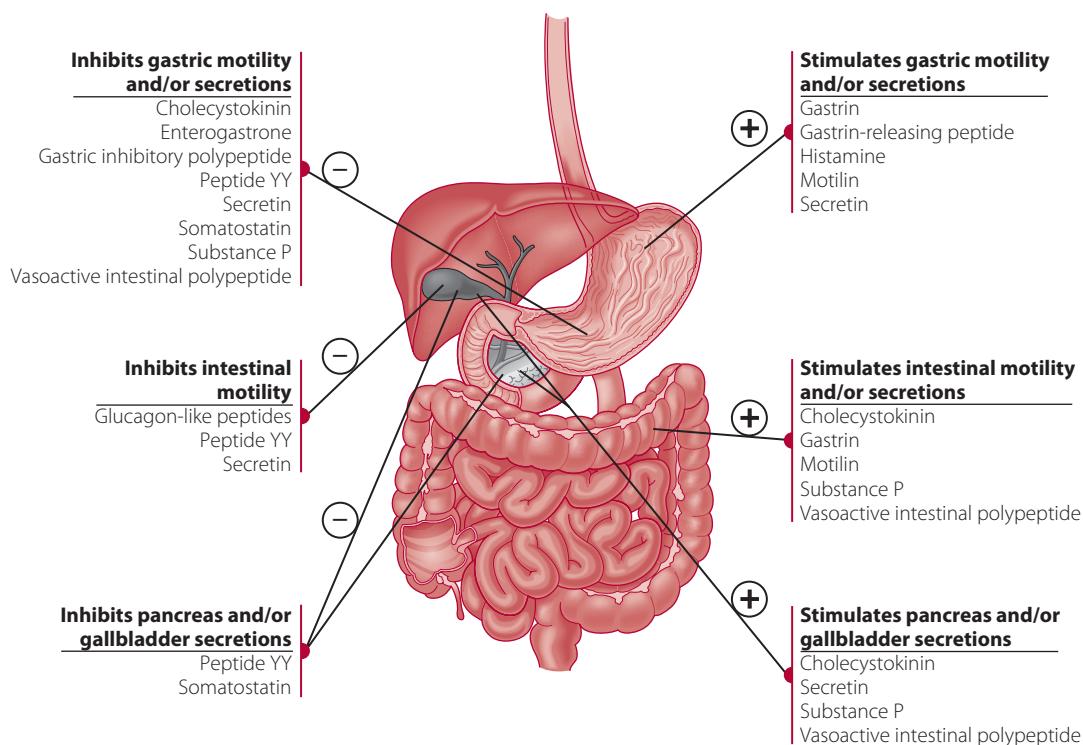


Figure 2.8 The effects of selected gastrointestinal hormones/peptides on gastrointestinal tract secretions and motility.

potent secretagogues (compounds that stimulate secretion) of hydrochloric acid release by the parietal cells include:

- gastrin, which acts on parietal cells in the stomach
- acetylcholine, released from the vagus nerve for action on parietal cells
- histamine, released from gastrointestinal tract mast (enterochromaffin) cells, which binds to H₂ receptors on parietal cells

Selected Diseases and Conditions of the Stomach

Understanding how hydrochloric acid is produced in the body and what stimulates its release is essential to understanding the treatment of peptic ulcers. Peptic ulcers typically result when the normal defense and repair systems that protect the mucosa of the gastrointestinal tract are disrupted. The most common cause of peptic ulcers is the bacterium *Helicobacter pylori*; however, chronic use of many substances, including aspirin, alcohol, and nonsteroidal anti-inflammatory drugs (NSAID) like ibuprofen, can disrupt the mucus-rich and bicarbonate-rich barriers that protect the mucosa and deeper layers of the gastrointestinal tract and can promote the development of ulcers. Several drugs used to treat peptic ulcers—cimetidine (Tagamet), ranitidine (Zantac), famotidine (Pepcid), and nizatidine (Axid)—prevent histamine from binding to H₂ receptors on parietal cells. These drugs, known as H₂ receptor blockers, bind to the H₂ receptors on the parietal cells. When histamine is released, it cannot bind to the H₂ receptor (the drug blocks histamine's ability to bind), and acid release from the parietal cell is diminished. Other drugs used to treat ulcers—omeprazole (Prilosec) and esomeprazole (Nexium)—work by binding to the ATPase/proton pump (Figure 2.6) at the secretory surface of the parietal cell and thus directly inhibit hydrogen release into the gastric juice. Drug therapies are quite effective in treating peptic ulcers; however, foods that irritate the gastric mucosa also must be avoided during acute peptic ulcer episodes. If a peptic ulcer results in bleeding into the gastrointestinal tract, further medical nutrition therapy may require increased consumption of nutrients such as protein and iron.

Regulation of Gut Motility and Gastric Emptying

When food is swallowed, the proximal portion of the stomach relaxes to accommodate the ingested food. The relaxation, considered to be a reflex, is controlled by two processes mediated by the vagus nerve: receptive relaxation and gastric accommodation. Signals for antral contraction (necessary for gastric emptying) occur at regular intervals and begin in the proximal stomach at a point along the greater curvature. The signals then migrate distally toward the pyloric sphincter at the juncture of the stomach and the small intestine. The pacemaker, located between the fundus and body of the stomach (Figure 2.4), signals the antrum. The pacemaker determines the frequency of the contractions

that occur. As the food moves into the antrum, the rate of contractions increases so that in the distal portion of the stomach food is liquefied into chyme. The rate of contractions is about 3 per minute in the stomach and increases to about 8 to 12 per minute in the proximal small intestine. The rate per minute decreases slightly to about 7 per minute in the distal small intestine.

The migrating motility or myoelectric complex, a series of contractions with several phases, moves distally like a wave down the gastrointestinal tract, but mainly in the stomach and intestine. The migrating motility complex waves occur approximately every 80 to 120 minutes during interdigestive periods, but their frequency changes during digestive periods. The migrating motility complex sweeps out gastrointestinal (especially gastric and intestinal) contents and prevents bacterial overgrowth in the intestine. Its activity is influenced by a variety of factors, including hormones and peptides. For example, the peptide motilin, secreted by cells of the duodenum, causes intestinal smooth muscle to contract and may be involved in regulating different phases of the migrating motility complex.

Gastric emptying is also influenced by several other factors. Receptors in the duodenal bulb (the first few centimeters of the proximal duodenum) are sensitive to the volume of chyme and to the osmolarity of the chyme present in the duodenum. Large volumes of chyme, for example, result in increased pressure within the stomach and promote gastric emptying. The presence of hypertonic/hyperosmolar (very concentrated) or hypotonic/hyposmolar (very dilute) chyme in the duodenum activates osmoreceptors. Activation of the osmoreceptors in turn slows gastric emptying, to facilitate the formation of chyme that is isotonic. In addition to volume and osmolarity, the chemical composition of the chyme also affects gastric emptying. Carbohydrate-rich and protein-rich foods appear to empty at about the same rate from the stomach; high-fat foods, however, slow gastric emptying into the duodenum. Salts and monosaccharides also slow gastric emptying, as do many free amino acids, such as tryptophan and phenylalanine, and complex carbohydrates, especially soluble fiber. The presence of acid in the duodenum stimulates the secretion of hormones and regulatory peptides that, along with some reflexes, also influence gastric emptying. For example, hormones such as secretin, glucose-dependent insulinotropic peptide (GIP), somatostatin, peptide YY, and enterogastrone decrease or inhibit gastric motility, as does the ileogastric reflex.

Although contractions within the stomach promote physical disintegration of solid foods into liquid form, complete liquefaction is not necessary for the stomach contents to empty through the pyloric sphincter into the duodenum. Particles as large as 3 mm in diameter ($\sim\frac{1}{8}$ in.) can be emptied from the stomach through the sphincter, but solid particles are usually emptied with fluids when

they have been degraded to a diameter of about 2 mm or less. Approximately 1 to 5 mL (~1 tsp) of chyme enters the duodenum about twice per minute. Contraction of the pylorus and proximal duodenum is thought to be coordinated with contraction of the antrum to facilitate gastric emptying. Gastric emptying following a meal usually takes between 2 and 6 hours.

THE SMALL INTESTINE

Once through the pyloric sphincter, chyme enters the small intestine. The small intestine (Figure 2.9), which represents the main site for nutrient digestion and absorption, is composed of the duodenum (slightly less than 1 ft long) and the jejunum and ileum (which together are approximately 9 ft long). Microscopy is generally needed to identify where one of these sections of the small intestine ends and the other begins. However, the Treitz ligament, a suspensory ligament, is found at about the site where the duodenum and jejunum meet. The lumen of the jejunum is generally larger than that of the ileum.

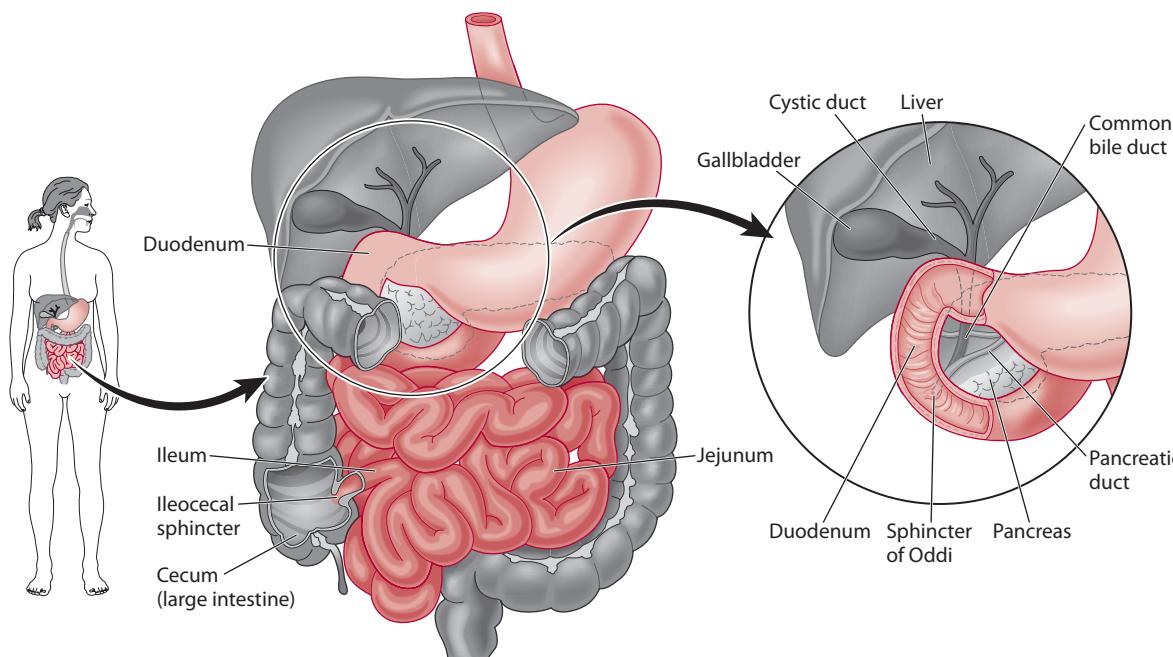
Structural Aspects of the Small Intestine

Although the structure of the small intestine consists of the same layers identified in Figure 2.2, the epithelial lining or mucosa of the small intestine is structured to maximize

surface area and thus maximize its ability to absorb nutrients. The small intestine has a surface area of approximately 300 m^2 , an area about equal to a 3-ft-wide sidewalk more than three football fields in length. Several structures, shown in Figure 2.10, that contribute to this enormous surface area include:

- large circular folds of mucosa, called the folds of Kerckring, that protrude into the lumen of the small intestine
- fingerlike projections, called villi, that project out into the lumen of the intestine and consist of hundreds of cells (**enterocytes**, also called absorptive epithelial cells) along with blood capillaries and a central lacteal (lymphatic vessel) for transport of nutrients out of the enterocyte
- microvilli, hairlike extensions of the plasma membrane of the enterocytes that make up the villi

The microvilli possess a surface coat, or glycocalyx, as shown in Figure 2.11; together, these make up the brush border of the enterocytes. Covering the brush border is an unstirred water (fluid) layer. That is, the unstirred water layer lies between the brush border membrane of the intestine and the intestinal lumen. Its presence can have the greatest effect on lipid absorption, as discussed in Chapter 5.



The small intestine is divided into 3 regions: the duodenum, jejunum, and ileum. The ileocecal sphincter regulates the flow of material from the ileum, the last segment of the small intestine, into the cecum, the first portion of the large intestine.

The duodenum receives secretions from the gallbladder via the common bile duct. The pancreas releases its secretions into the pancreatic duct, which eventually joins the common bile duct. The sphincter of Oddi regulates the flow of these secretions into the duodenum.

Figure 2.9 The small intestine.

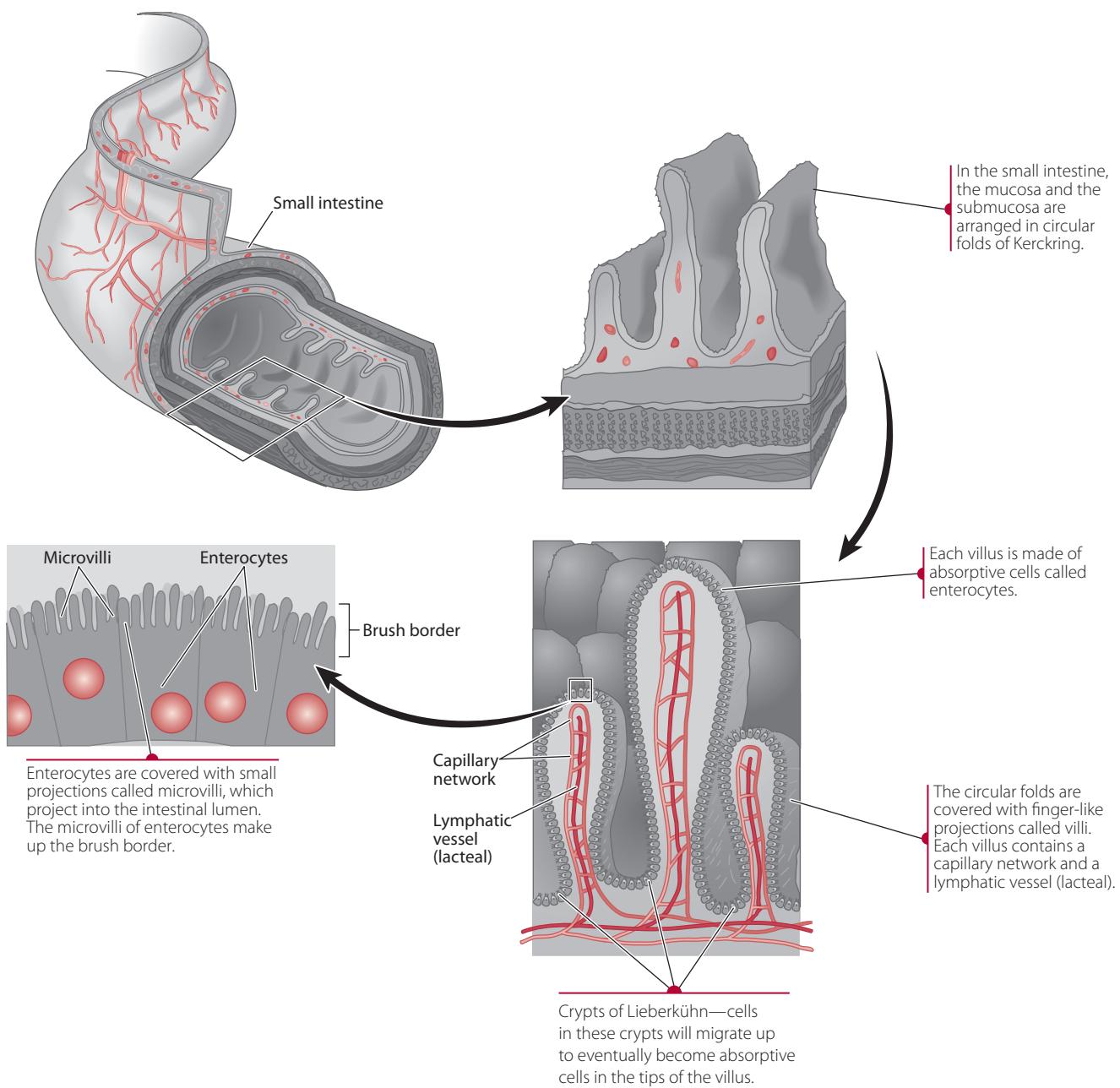


Figure 2.10 The structure of the small intestine.

Most of the digestive enzymes produced by the intestinal mucosal cells are found embedded on the brush border, and they hydrolyze already partially digested nutrients, mainly carbohydrate and protein (Table 2.1). Structurally, the digestive enzymes are glycoproteins. The carbohydrate (glyco) portion of these glycoprotein enzymes may in part make up the glycocalyx. The glycocalyx, which lines the luminal side of the intestine, is thought to consist of numerous fine filaments that extend almost perpendicular from the microvillus membrane to which it is attached. Digestion of nutrients is usually completed on the brush border but may be completed within the cytoplasm of the enterocytes. More detailed

information on carbohydrate, fat, and protein digestion is provided in Chapters 3, 5, and 6.

The small intestine also contains small pits or pockets called crypts of Lieberkühn (Figure 2.10) that lie between the villi. Epithelial cells in these crypts continuously undergo mitosis. The new cells gradually migrate upward and out of the crypts toward the tips of the villi. Toward the tip, many former crypt cells function as absorptive enterocytes. Ultimately, the enterocytes are sloughed off into the intestinal lumen and excreted in the feces. Intestinal cell turnover is rapid, approximately every 3 to 5 days. Cells in the crypts include Paneth cells that secrete antimicrobial peptides (called defensins) with broad activity,

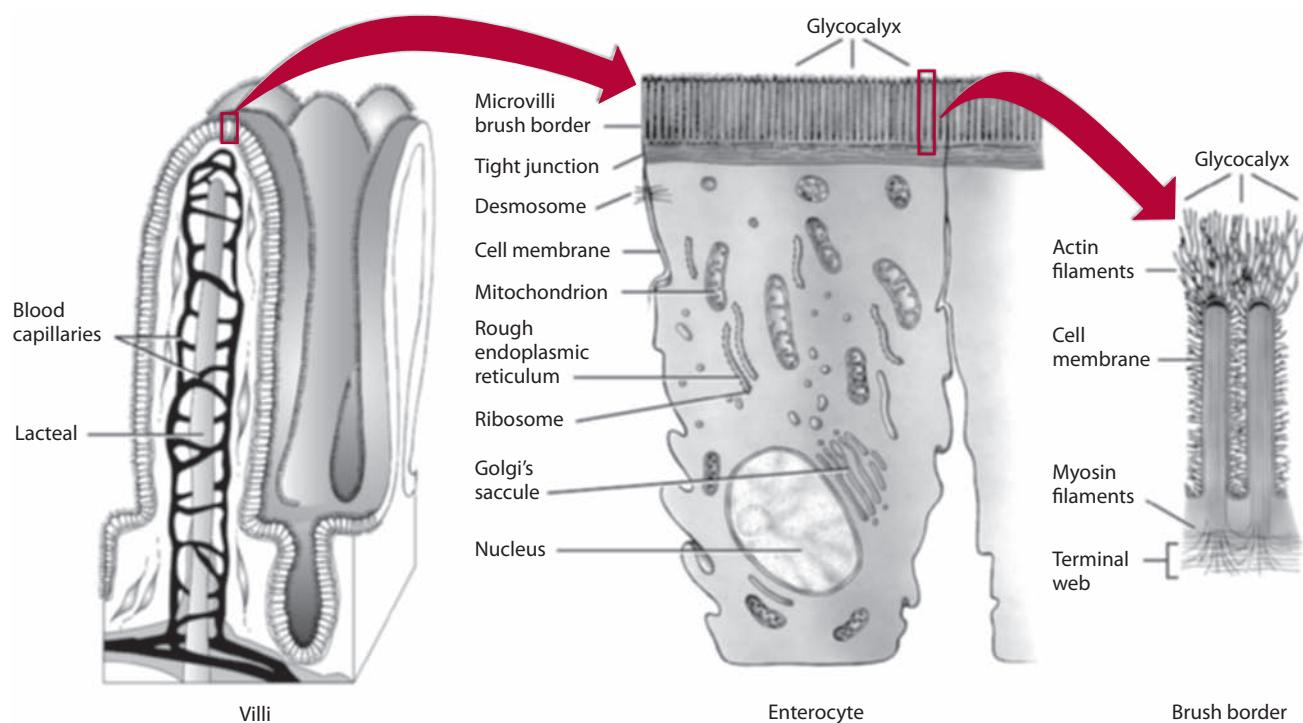


Figure 2.11 Structure of the absorptive cell of the small intestine.

enterochromaffin (mast) cells with endocrine functions, and goblet cells that secrete both small cysteine-rich proteins with antifungal activity and mucus. Mucus adheres to the mucosa and acts as a barrier to protect the epithelial mucosal cell surface from the acid chyme. Cells and glands in the crypts of Lieberkühn also secrete large volumes of intestinal juices and electrolytes into the lumen of the small intestine to facilitate nutrient digestion. Much of this fluid is typically reabsorbed by the villi.

Chyme moves through the small intestine propelled by various contractions influenced by the nervous system (Figure 2.12). Contractions of longitudinal smooth muscles, often called sleeve contractions, mix the intestinal contents with the digestive juices. Standing contractions of circular smooth muscles, called segmentation, produces bidirectional flow of the intestinal contents, occurs many times per minute, and mixes and churns the chyme with digestive secretions in the small intestine. Peristaltic waves, or progressive contractions, also accomplished primarily through action of the circular muscles, move the chyme distally along the intestinal mucosa toward the ileocecal valve.

Chyme moving from the stomach into the duodenum initially has a pH of about 2 because of its gastric acid content. The duodenum is protected against this gastric acidity by secretions from the Brunner's glands and from the pancreas. The Brunner's glands are located in the mucosa and submucosa of the first few centimeters of the duodenum (duodenal bulb). The mucus-containing secretions are viscous and alkaline, with a pH of approximately 8.2 to 9.3.

The mucus itself is rich in glycoproteins and helps protect the epithelial mucosa from damage. The pancreatic secretions released into the duodenum are rich in bicarbonate, which helps to neutralize the acid released from the stomach. Disruptions or inadequate release of these alkaline-rich secretions or excessive gastric acid secretion into the duodenum can precipitate the development of duodenal ulcers, which typically form around the duodenal bulb. As with gastric peptic ulcers, medications to suppress acid production are helpful in treating duodenal ulcers.

Regulation of Intestinal Secretions and Motility

Several hormones and peptides influence the release of intestinal secretions as well as intestinal motility. For example, vasoactive intestinal polypeptide (VIP), present in neurons within the gut, has been shown to stimulate intestinal secretions and relax most gastrointestinal sphincters. The neuropeptide substance P, the peptide motilin, and, to a lesser extent, the hormone cholecystokinin (CCK, also called CCK-pancreozymen and abbreviated CCK-PZ) increase intestinal motility. Conversely, motility of the intestine is inhibited or diminished by peptide YY, secretin, and glucagon-like peptides.

Immune System Protection of the Gastrointestinal Tract

A variety of immune system cells and lymphoid tissue protects the digestive tract. The lymphoid tissue is found primarily in the mucosa (especially the lamina propria)

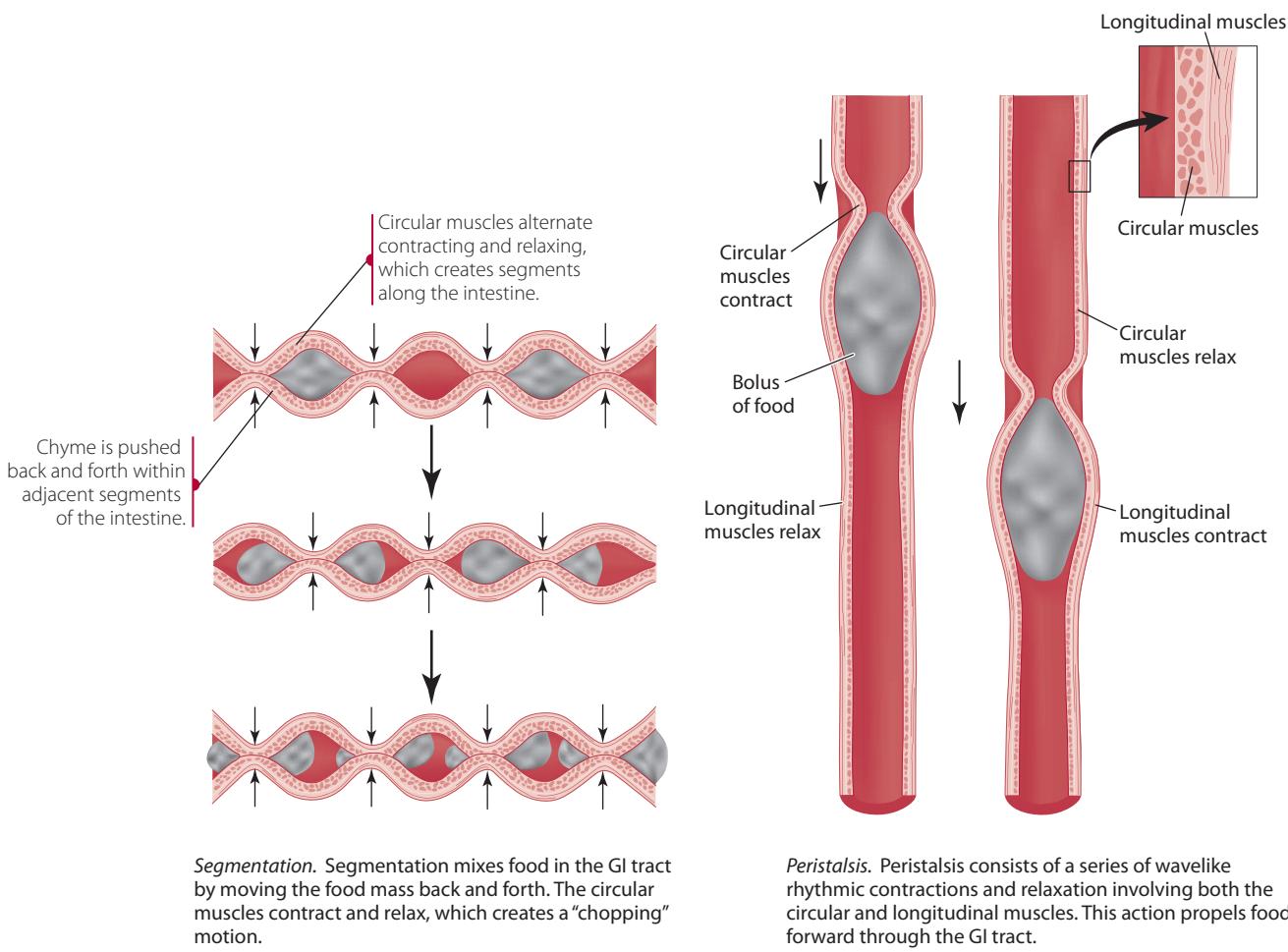


Figure 2.12 Movement of chyme in the gastrointestinal tract.

and submucosa and is called mucosa-associated lymphoid tissue (MALT) or, if found in the nonmucosal layer of the gastrointestinal tract, gut-associated lymphoid tissue (GALT). Both MALT and GALT are composed of multiple types of cells including T- and B-lymphocytes, plasma cells, natural killer (NK) cells, and macrophages, among others. The leukocytes tend to be located between the intestinal epithelial cells and make up approximately 15% of the epithelial mucosa. The majority of the body’s plasma cells are found in the lamina propria. The plasma cells produce secretory IgA, which binds antigens ingested with foods, inhibits the growth of pathogenic bacteria, and inhibits bacterial translocation. In addition to these cells, microfold (M) cells are associated with some lymphocytes and cover or overlie, usually in a single layer, Peyer patches. Peyer patches are aggregates of lymphoid tissue that also are located in the mucosa and submucosa. The M-cells pass or transport foreign antigens to the MALT lymphocytes, which in turn mount an immune response. After processing the foreign antigens, some of these lymphocytes are released from the Peyer patches and enter circulation to augment the immune response. Dendritic

cells, a type of macrophage, also are found in the gastrointestinal tract. They destroy foreign antigens and stimulate lymphocytes to destroy antigens.

Although the gastrointestinal tract provides a defense against bacteria and other foreign substances that may have been ingested with consumed food, this barrier can be easily destroyed. Atrophy of the mucosal and submucosal layers of the gastrointestinal tract, which may occur with illness, injury, starvation, or extended periods with little food intake, can lead to bacterial translocation. Bacterial translocation (the presence of gastrointestinal tract-derived bacteria or their toxins in the blood or lymph) can result in sepsis (infection) and, potentially, multiple system organ failure.

THE ACCESSORY ORGANS

Three organs, the pancreas, liver, and gallbladder, facilitate digestive and absorptive processes in the small intestine. The next section of this chapter describes each of these organs and its role in nutrient digestion, absorption, or both.

The Pancreas

The pancreas is a slender, elongated organ that ranges in length from about 6 to 9 inches. The pancreas is found behind the greater curvature of the stomach, lying between the stomach and the duodenum (Figures 2.1 and 2.13). Two types of active cells are found in the pancreas (Figure 2.13b):

- ductless endocrine cells that secrete hormones, primarily insulin and glucagon, into the blood
- acinar exocrine cells that produce the digestive enzymes, which get packaged in secretory structures called granules and released by **exocytosis** into pancreatic juice

Pancreatic juice, also produced by the acinar cells, contains:

- bicarbonate, important for neutralizing the acid chyme passing into the duodenum from the stomach and for maximizing enzyme activity within the duodenum
- electrolytes, including the cations sodium, potassium, and calcium and the anion chloride
- pancreatic digestive enzymes in a watery solution

To facilitate the release of pancreatic juice, the acinar cells of the pancreas are arranged into circular glands that are attached to small ducts. The pancreatic juice is secreted into the small ducts within the pancreas. These small ducts coalesce to form a large main pancreatic duct (Wirsung duct), which later joins with the common bile duct at the greater duodenal papilla, also called the ampulla of Vater,

to form the bile pancreatic duct. The bile pancreatic duct empties into the duodenum through the sphincter of Oddi (Figure 2.13a). Blockage of this duct, as may occur with gallstones from the gallbladder, may impair the release of pancreatic juice out of the pancreas and can lead to acute pancreatitis (inflammation of the pancreas), a potentially life-threatening condition. Pancreatitis is described in the Perspective at the end of this chapter.

Regulation of Pancreatic Secretions Pancreatic juice is released when pancreatic acinar cells are stimulated by hormones and the parasympathetic nervous system. The hormone secretin, secreted into the blood by enteroendocrine S-cells found in the mucosa of the proximal small intestine, is secreted in response to the release of acid chyme into the duodenum. Secretin stimulates the pancreas to secrete water, bicarbonate, and pancreatic enzymes. In addition to secretin, cholecystokinin secreted by enteroendocrine I-cells of the proximal small intestine, enteric nerves, and the neuropeptide substance P stimulate the secretion of pancreatic juices and enzymes into the duodenum. Similarly, vasoactive intestinal polypeptide (VIP), present in neurons within the gut, also stimulates pancreatic bicarbonate release into the small intestine. In contrast, somatostatin, which works in a paracrine fashion, inhibits pancreatic exocrine secretions. A variety of other gastrointestinal-derived hormones and peptides affect pancreatic insulin release including amylin, galanin, and somatostatin (inhibitory) and glucose-dependent insulinotropic polypeptide and glucagon-like peptide (stimulatory).

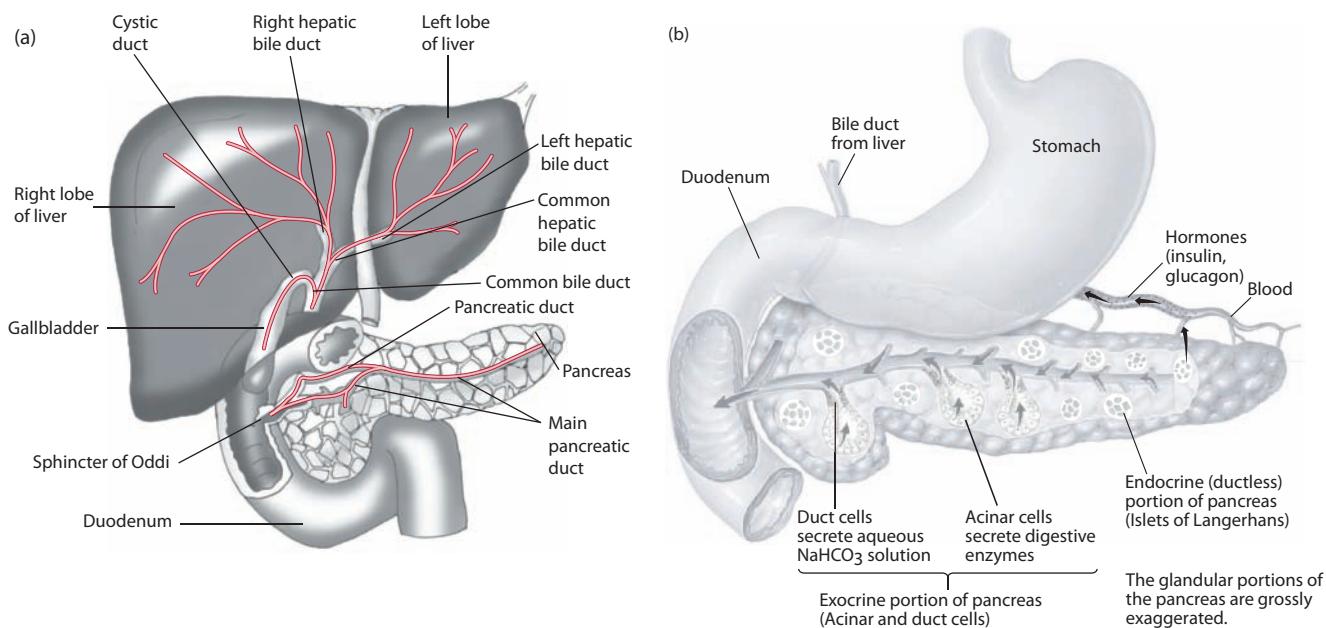


Figure 2.13 (a) The ducts of the gallbladder, liver, and pancreas. (b) Schematic representation of the exocrine and endocrine portions of the pancreas.

Pancreatic Digestive Enzymes The enzymes released by the pancreas, listed in Table 2.1, digest approximately half (50%) of all ingested carbohydrates, half (50%) of all proteins, and almost all (80% to 90%) of ingested fat. Proteases—enzymes that digest proteins—found in pancreatic juice and secreted into the duodenum include trypsinogen, chymotrypsinogen, procarboxypeptidases, proelastase, and collagenase. As a group, proteases hydrolyze peptide bonds, either internally or from the ends, and the net result of their collective actions is the production of polypeptides shorter in length than the original polypeptide or protein, oligopeptides (typically 4–10 amino acids in length), tripeptides, dipeptides, and free amino acids. The latter three may be absorbed into the enterocyte. Oligopeptides and some tripeptides may be further hydrolyzed by brush border aminopeptidases before being absorbed. More detailed information on protein digestion is given in Chapter 6. Only one enzyme, pancreatic α amylase, is secreted by the pancreas into the duodenum for carbohydrate digestion; carbohydrate digestion is covered in detail in Chapter 3. Enzymes necessary for lipid digestion produced by the pancreas include pancreatic lipase, the major fat digesting enzyme, and colipase. These enzymes and fat digestion are described in detail in Chapter 5.

The Liver

Another accessory organ to the gastrointestinal tract is the liver, pictured in Figures 2.1, 2.13, and 2.14. The liver, the largest single internal organ of the body, is made up of two lobes, the right lobe and left lobe. These lobes in turn contain functional units called lobules. The lobules (Figure 2.14) are made up of plates or sheets of liver cells, also called hepatocytes. The plates of cells are arranged so that they radiate out from central veins. Thus, the liver has multiple plates of cells radiating from multiple central veins. The central veins direct blood from the liver into general circulation through hepatic veins and then ultimately into the inferior vena cava. Blood passes between the plates of liver cells by way of sinusoids, which function like a channel and arise from branches of the hepatic artery and from the portal vein. The portal vein takes blood rich in nutrients away from the digestive tract and pancreas to the liver. Sinusoids allow blood from these two blood vessels (the portal vein and the hepatic artery) to mix and also enable uptake of nutrients through the endothelial cells that line the sinusoids. Sinusoids also contain macrophages called Kupffer's cells, which phagocytize bacteria and other foreign substances and thus serve to protect the body. Bile canaliculi lie between the hepatocytes in the hepatic plates. Bile, covered in the section on the gallbladder, drains from the canaliculi into bile ducts. As shown in Figure 2.13, the right and left hepatic bile ducts join to form the common hepatic duct. The common hepatic duct unites with the cystic duct from the gallbladder to form the common hepatic bile duct.

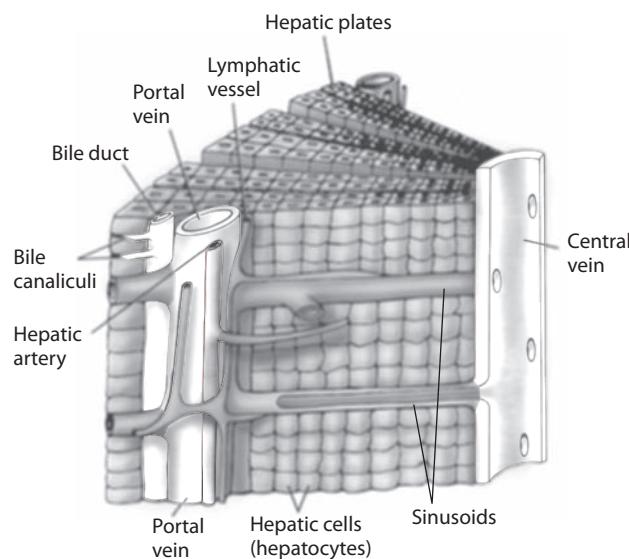


Figure 2.14 Structure of a liver lobule.

The Gallbladder

The gallbladder, a small organ with a capacity of approximately 40 to 50 mL (1.4–1.8 oz), is located on the surface of the liver (Figures 2.9 and 2.13). The gallbladder concentrates and stores the bile made in the liver until it is needed for fat digestion in the small intestine. The hormone cholecystokinin, secreted into the blood by enteroendocrine cells (called I-cells) of the proximal small intestine, stimulates the gallbladder to contract and release bile into the duodenum. In contrast, somatostatin, which works in a paracrine fashion, inhibits gallbladder contraction. Bile flow into the duodenum is regulated by the intraduodenal segment of the common hepatic bile duct and the sphincter of Oddi, located at the junction of the common hepatic bile duct and the duodenum (Figure 2.13). Bile synthesis, storage, function in fat digestion, recirculation, and excretion are described in the next sections.

Bile Synthesis Bile is a greenish-yellow fluid composed mainly of bile acids and salts but also cholesterol, phospholipids, and bile pigments (bilirubin and biliverdin) dissolved in an alkaline solution. The bile acids are synthesized in the hepatocytes from cholesterol, which in a series of reactions is oxidized to chenodeoxycholic acid and cholic acid, the two principal or primary bile acids (see Chapter 5 for further details). Chenodeoxycholate and cholate, once formed, conjugate primarily (~75%) with the amino acid glycine to form the conjugated bile acids glycochenodeoxycholic acid (glycochenodeoxycholate) and glycocholic acid (glycocholate), respectively.

Bile Acid	Amino Acid	Conjugated Bile Acid
Cholic acid	+ glycine	→ Glycocholic acid
Chenodeoxycholic acid	+ glycine	→ Glycochenodeoxycholic acid

Alternately and to a lesser (25%) extent, chenodeoxycholate and cholate conjugate with the amino acid taurine to form two additional primary conjugated bile acids.

Bile Acid	Amino Acid	Conjugated Bile Acid
Cholic acid	+ taurine	Taurocholic acid
Chenodeoxycholic acid	+ taurine	Taurochenodeoxycholic acid

Conjugation of the bile acids with these amino acids results in better ionization and thus in improved ability to form micelles. The formation and role of micelles in fat digestion are discussed in Chapter 5. Chenodeoxycholate and cholate are primary bile acids and make up 80% of the body's total bile acids. The remaining 20% of the bile acids is made up of secondary products produced in the large intestine from bacterial action on chenodeoxycholic acid to form lithocholate and on cholic acid to form deoxycholate. In addition to being conjugated to amino acids, most conjugated bile acids are present in bile as bile salts owing to bile's pH (~7.6–8.6). Sodium is the predominant biliary cation, although potassium and calcium bile salts may also be found in the alkaline bile solution.

Although bile acids and salts make up a large portion of bile, other substances are also found in bile. These other substances include both cholesterol and phospholipids, especially lecithin, and make up what is referred to as the bile acid-dependent fraction of bile. In addition, water, electrolytes, bicarbonate, and glucuronic acid conjugated bile pigments (mainly bilirubin, biliverdin, or both—waste end products of hemoglobin degradation that are excreted in bile and give bile its color) are secreted into bile by hepatocytes. This alkaline-rich fraction of the bile is referred to as bile acid-independent. The bile components must remain in the proper ratio to prevent gallstone formation (cholelithiasis), although other factors influence gallstone production.

Selected Conditions/Diseases of the Gallbladder Gallstones are thought to form when bile becomes supersaturated with cholesterol. Cholesterol precipitates out of solution and provides a crystalline-like structure within which calcium, bilirubin, phospholipids, and other compounds deposit, ultimately forming a “stone.” Gallstones may reside silently in the gallbladder, or they may irritate the organ, causing cholecystitis (inflammation of the gallbladder), or lodge in the common bile duct, blocking the flow of bile (choledocholithiasis) into the duodenum. Gallstones also may block the pancreatic duct, causing pancreatitis (inflammation of the pancreas), as described in the Perspective at the end of this chapter.

Bile Storage During the interdigestive periods, bile is sent from the liver to the gallbladder, where it is concentrated and stored. The gallbladder concentrates the bile so that as much as 90% of the water, along with some of the electrolytes, is reabsorbed by the gallbladder mucosa. The fluid

reabsorption thus leaves the remaining bile constituents (i.e., bile acids and salts, cholesterol, lecithin, bilirubin, and biliverdin) in a less dilute form. Concentration of the bile permits the gallbladder to store more of the bile produced by the liver between periods of food ingestion. Cholecystokinin, released in response to chyme entering the duodenum, stimulates gallbladder contraction. Bile is secreted into the duodenum through the sphincter of Oddi.

The Function of Bile Bile acids and bile salts act as detergents to emulsify lipids, that is, to break down large fat globules into small (about 1 mm diameter) fat droplets. Bile acids and salts, along with phospholipids, help to absorb lipids by forming small (<10 nm) spherical, cylindrical, or disklike complexes called micelles. Micelles can contain as many as 40 bile salt molecules. More thorough coverage of the functions of bile is found in Chapter 5.

The Recirculation and Excretion of Bile The human body contains a total bile acid pool of about 2.5 to 5.0 g. Greater than 90% of the bile acids and salts secreted into the duodenum is reabsorbed by active transport in the ileum. Small amounts of the bile may be passively reabsorbed in the jejunum and the colon. About half of the cholesterol contained within the bile is taken up by the jejunum and used in forming chylomicrons (see Chapter 5). The remainder of the cholesterol is excreted. Bile that is absorbed in the ileum enters the portal vein and is transported, attached to the plasma protein albumin in the blood, back to the liver. Once in the liver, the reabsorbed bile acids are reconjugated to amino acids if necessary and secreted into bile along with the newly synthesized bile acids. New bile acids typically are synthesized in amounts about equal to those lost in the feces. New bile, mixed with recirculated bile, is sent through the cystic duct to be stored in the gallbladder. The circulation of bile, termed enterohepatic circulation, is pictured in Figure 2.15. The pool of bile is thought to recycle at least twice per meal.

Some of the bile acids that are not reabsorbed in the ileum may be deconjugated by bacteria in the colon and possibly the terminal ileum to form secondary bile acids (Figure 2.16). For example, cholic acid, a primary bile acid, is converted to the secondary bile acid deoxycholic acid, which can be reabsorbed. Chenodeoxycholic acid is converted to the secondary bile acid lithocholic acid, which unlike deoxycholic acid typically is excreted in the feces. Certain dietary fibers present in the gastrointestinal tract may bind to the bile salts and acids, however, and prevent bacterial deconjugation and conversion to secondary bile acids. About 0.5 g of bile salts are lost daily in the feces.

Bile Circulation and Hypercholesterolemia Knowing how bile is recirculated and is excreted helps in understanding the mechanisms by which various drug therapies

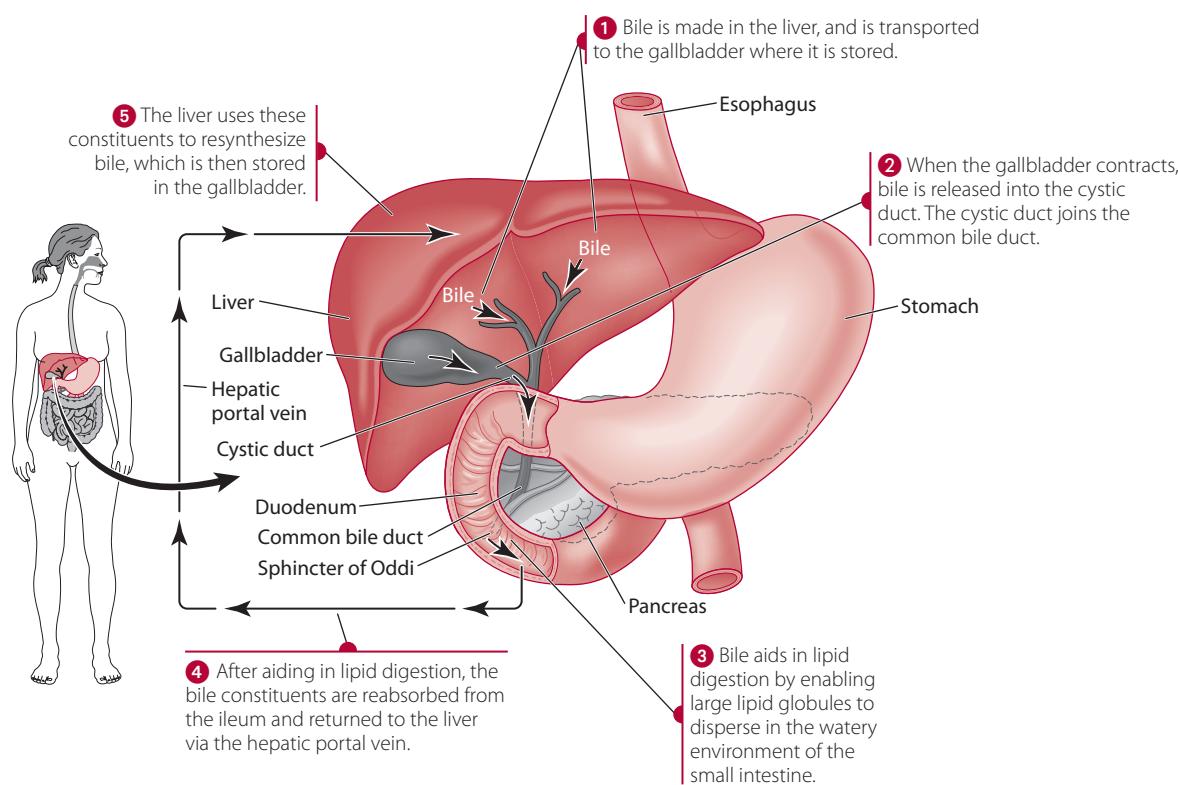


Figure 2.15 Enterohepatic circulation of bile.

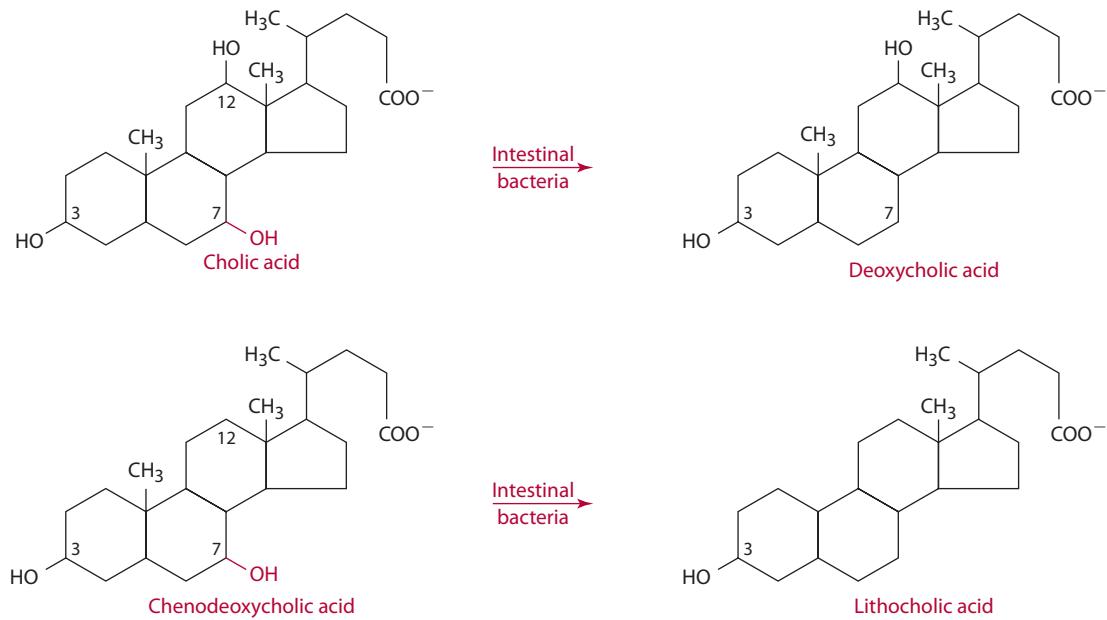


Figure 2.16 The synthesis of secondary bile acids by intestinal bacteria.

and functional foods help to treat high blood cholesterol concentrations (hypercholesterolemia). People with hypercholesterolemia are often given certain medications—specifically, resins such as cholestyramine (Questran). The function of the medications is to bind bile in the gastrointestinal tract and enhance its fecal excretion from the body.

In addition, some food manufacturers add plant (phyto) stanols and sterols to foods such as margarines, orange juice, and granola bars. These phytostanols and phytosterols bind bile as well as dietary and endogenous cholesterol in the gastrointestinal tract and enhance fecal excretion from the body. The increased fecal excretion of the bile,

decreased recirculation of the bile, and decreased absorption of cholesterol requires the body to use cholesterol to synthesize new bile acids. The increased use of cholesterol to make more bile diminishes the body's cholesterol concentrations. Thus, the goal of using such medications and functional foods is to lower blood cholesterol concentrations and reduce risk of cardiovascular disease. Health claims on the labels of some of the products containing phytosterols state that "Plant sterols, eaten twice a day with food for a total of 0.8 g daily total, may reduce heart disease risk in a diet low in saturated fat and cholesterol." Daily consumption of plant sterols has been shown to decrease total and LDL plasma cholesterol concentrations in people with normal blood lipid and high blood lipid concentrations.

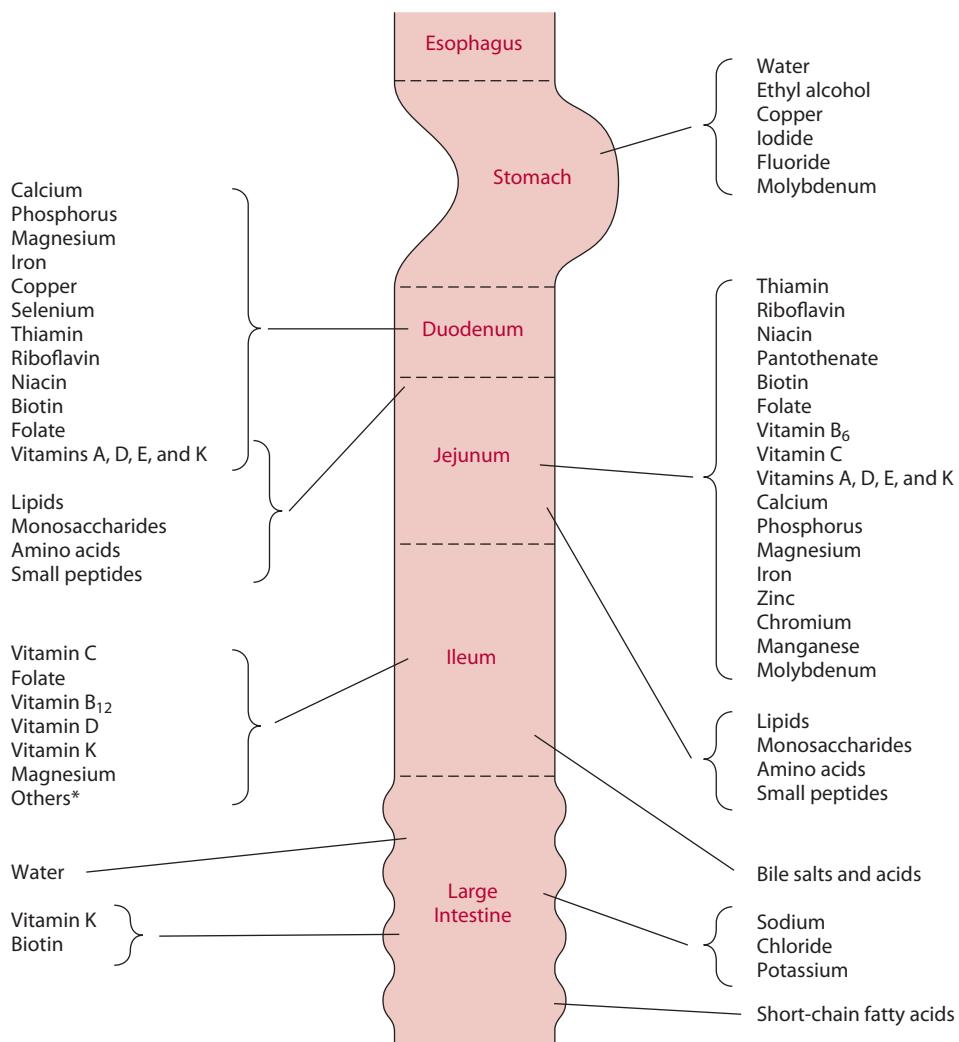
THE DIGESTIVE AND ABSORPTIVE PROCESSES

Most nutrients must be digested—that is, broken down into smaller pieces—before they can be absorbed. Nutrient

digestion occurs both in the lumen of the gastrointestinal tract and on the brush border and is accomplished through enzymes from the mouth, stomach, pancreas, and small intestine and with the help of bile from the liver. Once digested, nutrients must move into the cells of the gastrointestinal tract by a process known as absorption. Although some nutrient absorption may occur in the stomach, the absorption of most nutrients begins in the duodenum and continues throughout the jejunum and ileum, as shown in Figure 2.17. Generally, most absorption occurs in the proximal (upper) portion of the small intestine.

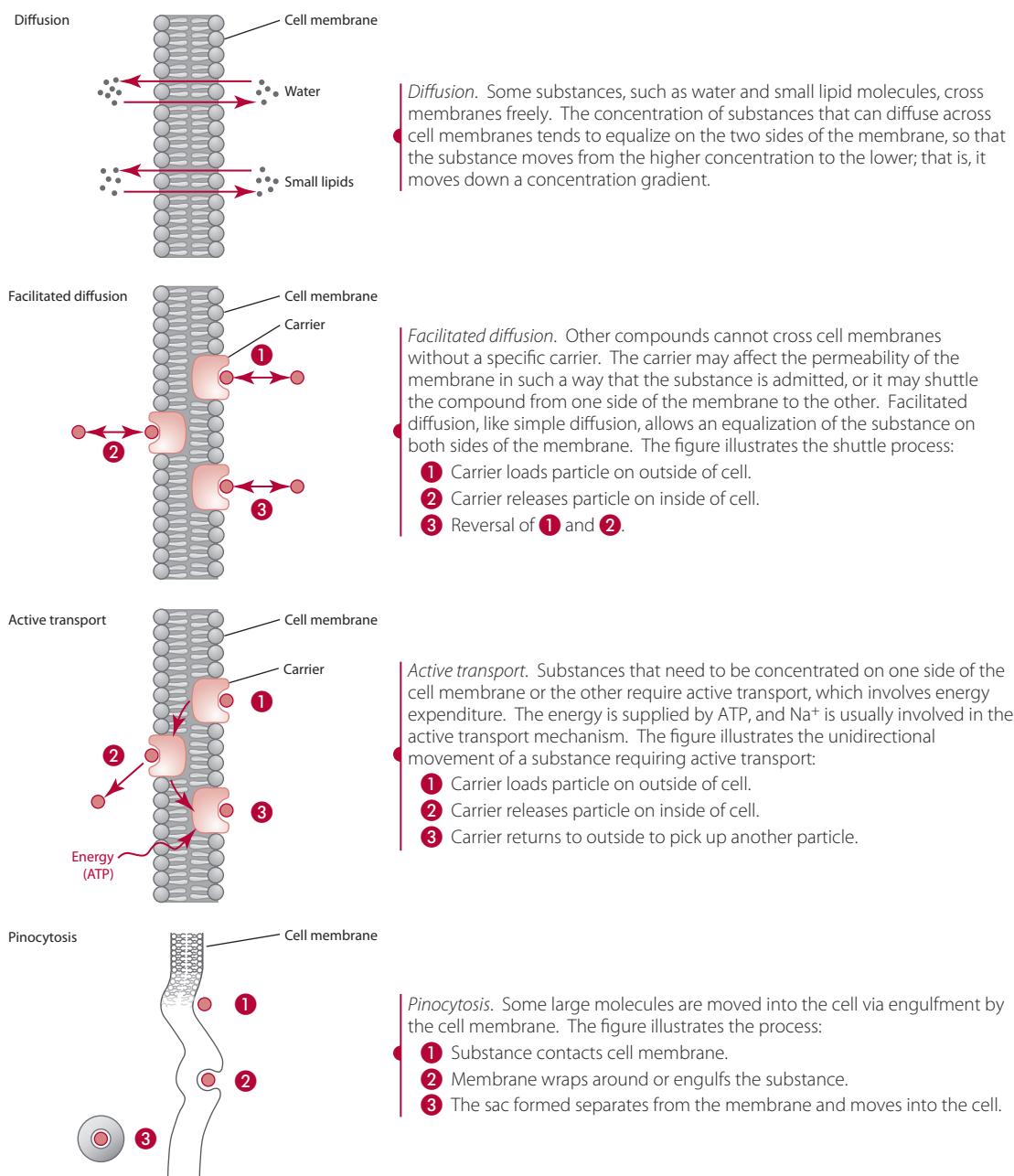
Digestion and absorption of nutrients within the small intestine are rapid, with most of the carbohydrate, protein, and fat being absorbed within 30 minutes after chyme has reached the small intestine. The presence of unabsorbed food in the ileum may increase the amount of time material remains in the small intestine and therefore may increase nutrient absorption.

Nutrients may be absorbed into enterocytes by diffusion, facilitated diffusion, active transport, or, occasionally,



*Many additional nutrients may be absorbed from the ileum depending on transit time.

Figure 2.17 Sites of nutrient absorption in the gastrointestinal tract.

**Figure 2.18** Primary mechanisms for nutrient absorption.

pinocytosis or **endocytosis** (Figure 2.18). In addition, a few nutrients may be absorbed by a paracellular (between-cells) route. The mechanism of absorption for a nutrient depends on several factors:

- solubility (fat versus water) of the nutrient
- concentration or electrical gradient
- size of the molecule to be absorbed

The absorption and transport of amino acids, peptides, monosaccharides, fatty acids, monoacylglycerols, and glycerol—that is, the end products of macronutrient

digestion—are considered in depth in Chapters 3, 5, and 6. The digestion and mechanisms of absorption for each of the vitamins and minerals are described in detail in Chapters 9 to 13; the sites of absorption are shown in Figure 2.17.

Unabsorbed intestinal contents are passed from the ileum through the ileocecal valve into the colon, although some may serve as substrates for bacteria that inhabit the small intestine. Bacterial counts in the small intestine range up to about 10^3 per gram of intestinal contents; counts may be even higher near the ileocecal sphincter. Examples of some of the bacteria that may be found in

the small intestine include bacteroides, enterobacteria, lactobacilli, streptococci, and staphylococci.

THE COLON OR LARGE INTESTINE

From the ileum (which is the distal or terminal section of the small intestine), unabsorbed materials empty through the ileocecal valve into the cecum, the right side of the colon (large intestine). From the cecum, materials move sequentially through the ascending, transverse, descending, and sigmoid sections of the colon (Figure 2.19). The colon in its entirety is almost 5 ft long and is larger in diameter than the small intestine, thus explaining the terminology distinction (large versus small) between the two intestines.

Rather than being a part of the entire wall of the digestive tract, as it is in the upper digestive tract, the longitudinal muscle in the colon is gathered into three muscular bands or strips called teniae (also spelled taenia or teneae) coli that extend throughout most of the colon. Contraction of a strip of longitudinal muscle, along with contraction of circular muscle, causes the uncontracted portions of the colon to bulge outward, creating pouches (haustra). Contractions typically occur in one area of the colon and then move to a different, nearby area.

On initially entering the colon, the intestinal material is still quite fluid. Contraction of the musculature of the large intestine is coordinated so as to mix the intestinal contents gently and to keep material in the proximal (ascending) colon a sufficient length of time to allow nutrients to be absorbed. As described by Guyton [1], the fecal material is slowly dug into and rolled over in the colon as one would spade the earth, so that deeper, moister fecal matter is put in contact with the colon's absorptive surface.

The proximal colonic epithelia absorb sodium, chloride, and water more effectively than does the small intestinal mucosa. For example, about 90% to 95% of the water and sodium entering the colon each day is absorbed. Colonic absorption of sodium is influenced by a number of factors,

including hormones. Antidiuretic hormone, for example, decreases sodium absorption, whereas glucocorticoids and mineralocorticoids increase sodium absorption in the colon.

Secretions into the lumen of the colon are few, but some nutrient exchange occurs. Goblet cells in the crypts of Lieberkühn secrete mucus. Mucus protects the colonic mucosa and acts as a lubricant for fecal matter. Potassium is secreted, possibly through an active secretory pathway, into the colon. Bicarbonate is also secreted in exchange for chloride absorption. Bicarbonate provides an alkaline environment that helps neutralize acids produced by colonic anaerobic bacteria. Sodium and hydrogen ion exchanges also occur, permitting electrolyte absorption.

The end result of the passage of material through the colon, which usually takes about 12 to 70 hours, is that the unabsorbed materials are progressively dehydrated. Typically, the approximately 1 L of chyme that enters the large intestine each day is reduced to less than about 200 grams of defecated material containing sloughed gastrointestinal cells, inorganic matter, water, small amounts of unabsorbed nutrients and food residue, constituents of digestive juices, and bacteria.

Intestinal Bacteria (Microflora), Pre- and Probiotics, and Disease

Both gram-negative and gram-positive bacteria strains, representing over 400 species of at least 40 genera, have been isolated from human feces. Although intestinal bacterial counts in the large intestine have been reported to be as high as 10^{12} per gram of gastrointestinal tract contents, bacteria are found throughout the gastrointestinal tract. The mouth contains mostly anaerobic bacteria. The stomach contains few bacteria because of its low pH, but some more acid-resistant bacteria that are present include lactobacillus and streptococcus. The proximal small intestine contains both aerobes and facultative anaerobes. Most bacteria found in the ileum and large intestine are anaerobes, including bacteroides, lactobacilli, and clostridia.

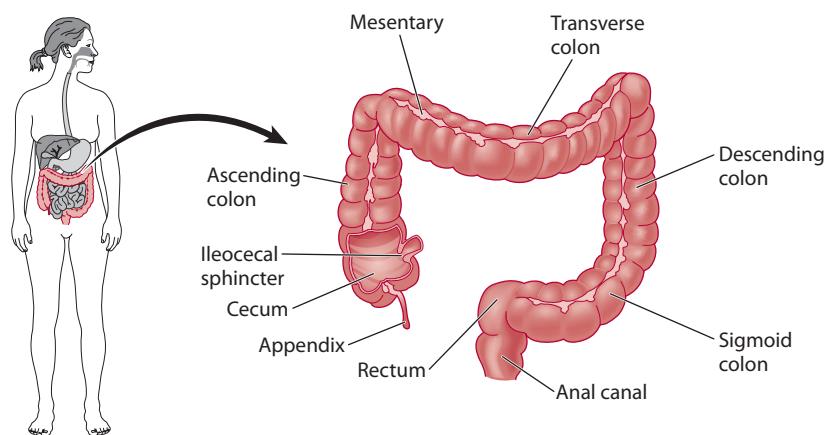


Figure 2.19 The colon.

Other examples of intestinal **microflora** (bacteria adapted to living in a specific environment) that inhabit the large intestine are bifidobacteria, methanogens, eubacteria, and streptococci. Anaerobic species are thought to outnumber aerobic species by at least 10 fold, but the exact composition of the microflora is affected by a variety of factors such as substrate availability, pH, medications, and diet, among others.

Bacteria gain nutrients for their own growth through unabsorbed food residues in the intestines. Bacteria use primarily dietary carbohydrate, and to a lesser extent amino acids and undigested protein, as substrates necessary for their growth. For example, starch that has not undergone hydrolysis by pancreatic amylase may be used by gram-negative bacteroides and by gram-positive bifidobacteria or eubacteria. Glycoproteins (mainly mucins) found in mucus secretions of the gastrointestinal tract may be broken down and used by bacteria such as bacteroides, bifidobacteria, and clostridia. In addition, sugar alcohols such as sorbitol and xylitol, disaccharides such as lactose, and some fibers such as some hemicelluloses, fructose oligosaccharides, pectins, and gums may be degraded by selected bacteria found in the colon. Digestive enzymes themselves may even serve as substrate for bacteria such as bacteroides and clostridia. The breakdown of carbohydrate and protein by bacteria is an anaerobic process referred to as **fermentation**.

As described above, bacteria degrade mostly carbohydrate but also some amino acids and protein as substrates for the production of substances, such as energy and carbon atoms, necessary for bacterial growth and maintenance. Acids are one of the principal end products of bacterial carbohydrate fermentation in the large intestine. Specifically, lactate and various short-chain fatty acids—acetate, butyrate, and propionate—are generated from bacterial action. These short-chain fatty acids, formerly called volatile fatty acids, serve many purposes. They are thought to stimulate gastrointestinal cell proliferation. The presence of the acids lowers the luminal pH in the colon to effect changes in nutrient absorption and in the growth of certain species of bacteria. In addition, these acids provide substrates for body cell use. Butyrate, for example, may be absorbed by a Na^+/H^+ or a K^+/H^+ exchange system in the colon, where it is a preferred energy source for colonic epithelial cells. Butyrate also may regulate gene expression and cell growth. Propionate and lactate are absorbed in the colon and taken up for use by liver cells. Acetate is absorbed and used by muscle and brain cells. Absorption of these acids appears to be concentration dependent.

In addition to the short-chain fatty acids, bacteria generate a number of other substances. For example, several different gases are produced by colonic bacteria, including methane (CH_4), hydrogen (H_2), hydrogen sulfide (H_2S), and carbon dioxide (CO_2). One estimate

suggests that colonic bacterial fermentation of about 10 g of carbohydrate can generate several liters of hydrogen gas. Much of the hydrogen and other gases that are generated can be used by other bacteria in the colon. Gases not used are excreted. Measurement of the hydrogen gas produced by bacteria is used as a basis to diagnose **lactose intolerance**, a condition in which the enzyme lactase is not made in sufficient quantities and thus is not available to digest the disaccharide lactose. Lactose intolerance is fairly common among adults, especially those of African American, American Indian, and Asian heritage. When someone with lactose intolerance ingests the sugar lactose (for example, by drinking milk), the lactose cannot be digested in the small intestine and enters the colon undigested. In the colon, the undigested lactose is fermented by colonic bacteria. These colonic bacteria, upon fermenting large quantities of lactose, in turn produce a lot more hydrogen gas than usual. Much of the hydrogen gas made by the bacteria is absorbed by the body and then exhaled in the breath. To diagnose lactose intolerance, a person usually is asked to consume about 50 g of lactose, and their breath is analyzed for hydrogen gas for the next several hours. Generally, if the person is lactose intolerant, hydrogen gas excretion in the breath increases about an hour to 1½ hours after lactose is consumed. An absence of an increase in breath hydrogen gas concentrations suggests adequate lactose digestion. Symptoms of lactose intolerance include bloating, gas (flatulence), and abdominal pain.

Amino acids also are degraded by bacteria. For example, bacterial degradation of the branched-chain amino acids generates branched-chain fatty acids such as isobutyrate and isovalerate. Deamination of aromatic amino acids (see Chapter 6) yields phenolic compounds. Amines such as histamine result from bacterial decarboxylation of amino acids such as histidine. Ammonia is generated by bacterial deamination of amino acids as well as by bacterial urease action on urea (secreted into the gastrointestinal tract from the blood). The ammonia can be reabsorbed by the colon and recirculated to the liver, where it can be reused to synthesize urea or amino acids. About 25%, or about 8 g, of the body's urea may be handled in this fashion. This process must be controlled in people with liver disease. High amounts of ammonia in the blood are thought to contribute to the development of hepatic encephalopathy and coma in people with liver disease (cirrhosis). Thus, low-protein diets are commonly needed in people with advanced liver disease. Uric acid and creatinine also may be released into the digestive tract and metabolized by colonic bacteria.

Probiotics (*pro* means “life” in Greek), foods that contain live cultures of specific strains of bacteria, are gaining interest in the health field. The intent of consuming probiotics is for the bacteria to survive the passage through the upper

digestive tract and then establish themselves in (colonize) the lower gastrointestinal tract, primarily the colon. At present, probiotics are mostly consumed as yogurt with live cultures or in fermented milk. In the United States, yogurt is often fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and milk is usually fermented by *L. acidophilus* and *L. casei*. Other bacteria used to manufacture dairy products include various species of leuconostoc and lactococcus. The most common probiotic bacteria are lactic acid bacteria such as bifidobacteria and lactobacilli.

In addition to probiotics, **prebiotics** also are being developed and promoted. Prebiotics are food ingredients that are not digested by human digestive enzymes but can benefit the host by acting as substrate for the growth and activity of one or more selected species of bacteria in the colon and thus improve the health of the host. For example, consuming various types of fiber such as oligosaccharides, beta-glucan, and pectin appears to effectively increase selected microbial populations, especially bifidobacteria and lactobacilli. The increased presence of these health-promoting bacteria in turn helps to inhibit the growth of other, pathogenic bacteria. Prebiotics and their uses are discussed in more detail in Chapter 4.

Probiotics appear to be beneficial in preventing and treating several conditions. Some of these conditions include diarrhea, inflammatory bowel diseases (Crohn's disease and ulcerative colitis), colon cancer, infected pancreatic necrosis, and postoperative liver transplant infections; however, with the exception of certain types of diarrheal illnesses, data are not sufficient to support routine probiotic use [2–7].

The mechanisms by which probiotics exert their effects are not clear, but some of the several hypotheses include both immunological and nonimmunological roles. Probiotics are generally thought to:

- enhance the host's immune defense system by increasing secretory IgA production, tightening the mucosal barrier, enhancing lymphocyte cytokine responses, and enhancing phagocytic activity, among other actions
- displace, exclude, or antagonize pathogenic bacteria from colonizing, for example, by competing for attachment sites on the intestinal mucosa or by strengthening the mucosal barrier to normalize intestinal permeability and to prevent pathogenic bacterial translocation
- acidify the colonic pH by producing fermentation products such as short-chain fatty acids
- transform and promote excretion of toxic substances such as bile acids, nitrosamines, heterocyclic amines, and mutagenic compounds
- enhance fecal bulk production, which may decrease (speed up) transit time and thereby lower the colon's exposure time to toxic substances [2–7]

Coordination and Regulation of the Digestive Process

NEURAL REGULATION

The sympathetic and parasympathetic nervous systems, as well as the enteric nervous system, mediate gastrointestinal activities. Sympathetic nervous system fibers, arising in the thoracic and lumbar regions of the spinal cord, innervate all areas of the gastrointestinal tract. Generally, norepinephrine, released from the nerve endings, acts on smooth muscle of the digestive tract to inhibit activity. Sympathetic efferent neurons, for example, decrease muscle contractions and constrict sphincters to diminish gastrointestinal motility. In contrast, the parasympathetic nervous system typically stimulates the digestive tract promoting motility (peristalsis), gastrointestinal reflexes, and secretions. For example, the facial and glossopharyngeal nerves stimulate saliva production, and the vagus nerve, which innervates the esophagus, stomach, pancreas, and proximal colon, stimulates gastric acid secretion, among other processes.

The nervous system of the gastrointestinal tract is referred to as the enteric (relating to the intestine) nervous system. The system includes millions of neurons and their processes embedded in the wall of the gastrointestinal tract beginning in the esophagus and extending to the anus. The enteric nervous system, which is connected to the central nervous system largely through the vagus nerve and other pathways out of the spinal cord, can be divided into two neuronal networks, or plexuses: the myenteric plexus (or plexus of Auerbach) and the submucosal plexus (or plexus of Meissner). The location and actions of these two plexuses are given here:

Myenteric Plexus	Submucosal Plexus
Lies in the muscularis externa between longitudinal and circular muscles of the muscularis propria	Lies in the submucosa (mostly in the intestines)
Controls peristaltic activity and gastrointestinal motility	Controls mainly gastrointestinal secretions and local blood flow

Impulses, either stimulatory or inhibitory, are sent from the enteric nervous system to the smooth circular and longitudinal muscles of the gastrointestinal tract. The myenteric plexus controls peristalsis, and when this plexus is stimulated, gastrointestinal activity generally increases. The submucosal plexus typically controls secretion release, receiving information from stretch receptors and gastrointestinal

epithelial cells in the intestinal wall. Also under control by the enteric nervous system is the regulation of gut motility by the migrating myoelectric or motility complex, described in the “Regulation of Gut Motility and Gastric Emptying” section of this chapter.

The enteric nervous system also affects gastrointestinal reflexes, called enterogastric reflexes. **Reflexes** are an involuntary response to stimuli and in the gastrointestinal tract affect secretions, blood flow, and peristalsis as well as other processes involved in digestion. Two examples of enterogastric reflexes include the ileogastric reflex and the gastroileal reflex. With the ileogastric reflex, gastric motility is inhibited when the ileum becomes distended. With the gastroileal reflex, ileal motility is stimulated when gastric motility and secretions increase. Other reflexes affect the small and large intestines. For example, the colonoileal reflex from the colon inhibits the emptying of the contents of the ileum into the colon. The intestinointestinal reflex diminishes intestinal motility when a segment of the intestine is overdistended.

REGULATORY PEPTIDES

Factors influencing digestion and absorption are coordinated, in part, by a group of gastrointestinal tract molecules called regulatory peptides or, more specifically, gastrointestinal hormones and neuropeptides. Regulatory peptides affect a variety of digestive functions, including gastrointestinal motility, intestinal absorption, cell growth, and the secretion of digestive enzymes, electrolytes, water, and other hormones.

Some of the regulatory peptides—such as gastrin, cholecystokinin, secretin, glucose-dependent insulinotropic peptide (GIP), and motilin—are considered hormones. In fact, many of the hormones can be categorized together (and called families) based on their amino acid sequences.

Some regulatory peptides are termed paracrines and neurocrines. When released by endocrine cells, paracrines

diffuse through extracellular spaces to their target tissues rather than being secreted into the blood (like hormones) for transport to target tissues. Some paracrines affecting the gastrointestinal tract include somatostatin, glucagon-like peptides, and insulin-like growth factor.

The functions of regulatory peptides with respect to the gastrointestinal tract and the digestive process are numerous and have been addressed to varying degrees in the sections on regulation of gastric and intestinal secretions and motility. Most, but not all, hormones and peptides have multiple actions; some are strictly inhibitory or stimulatory, whereas some mediate both types of responses. More than 100 regulatory peptides are thought to affect gastrointestinal functions. Table 2.2 summarizes some of the functions of a few of these peptides.

Gastrin, secreted into the blood primarily by enteroendocrine G-cells in the antrum of the stomach and proximal small intestine, acts mainly in the stomach. Gastrin release occurs in response to vagal stimulation, ingestion of specific substances or nutrients, gastric distention, hydrochloric acid in contact with gastric mucosa, and the effects of local and circulating hormones. Gastrin principally stimulates the release of hydrochloric acid, but it also stimulates gastric and intestinal motility and pepsinogen release. Gastrin also stimulates the cellular growth of (that is, has trophic action on) the stomach and both the small and large intestines.

Cholecystokinin (CCK), secreted into the blood by enteroendocrine I-cells of the proximal small intestine and by enteric nerves, principally stimulates secretion of pancreatic juice and enzymes into the duodenum. It also stimulates gallbladder contraction, which facilitates the release of bile into the duodenum, and to a limited extent, it stimulates gastric motility.

Secretin, secreted into the blood by enteroendocrine S-cells found in the proximal small intestine, is secreted in response to the release of acid chyme into the duodenum. Secretin acts primarily on pancreatic acinar cells to stimulate the release of pancreatic juice and enzymes into

Table 2.2 Selected Regulatory Hormones/Peptides of the Gastrointestinal Tract, Their Production Site, and Selected Functions

Hormone/Peptide	Production Sites (if known)	Some Selected Function(s)
Gastrin	Stomach and small intestine	Stimulates motility and gastric acid release
Cholecystokinin	Small intestine	Stimulates gall bladder contraction
Secretin	Small intestine	Stimulates pancreas juice and enzyme secretion
Motilin	Small intestine	Stimulates gastric and intestinal motility
Glucose-dependent Insulinotropic Peptide	Small intestine	Stimulates insulin secretion and inhibits gastric secretions and motility
Peptide YY	Small intestine	Inhibits motility and gastric and pancreatic secretions
Enterogastrone	Small intestine	Inhibits gastric secretions and motility
Amylin	Pancreas, stomach, small intestine	Inhibits gastric emptying
Somatostatin	Pancreas and small intestine	Inhibits gastric secretions and motility, and pancreatic and gall bladder secretions
Glucagon-like Peptides	Small and large intestines	Inhibits gastrointestinal tract motility
Substance P	Neurons and small intestine	Inhibits gastric secretions and stimulates intestinal motility

the intestine. Secretin is thought to stimulate pepsinogen release but inhibit gastric acid secretion. It may also inhibit motility of most of the gastrointestinal tract, especially the stomach and proximal small intestine.

Motilin, a peptide secreted by enteroendocrine M-cells of the duodenum and jejunum, stimulates gastric and duodenal motility, gastric and pancreatic secretions, and gallbladder contraction.

Glucose-dependent insulinotropic peptide (GIP), previously called gastric inhibitory peptide, is produced by enteroendocrine K-cells of the duodenum and jejunum and primarily stimulates insulin secretion. GIP also inhibits gastric secretions and motility.

Peptide YY, secreted by enteroendocrine cells of the ileum, inhibits gastric acid and pancreatic juice secretions and inhibits gastric and intestinal motility.

Enteroglucagon, secreted by the ileum, inhibits to a minor extent gastric and pancreatic secretions.

Amylin, secreted by the pancreatic cells and gastric and intestinal endocrine cells, delays gastric emptying and inhibits postprandial glucagon secretion.

Paracrine-acting substances usually work by entering secretions. Three examples of paracrine-acting peptides are:

- Somatostatin, synthesized by pancreatic Δ (D) cells and intestinal cells, appears to mediate the inhibition of gastrin release as well as the release of GIP, secretin, VIP, and motilin and thus inhibits gastric acid, gastric motility, pancreatic exocrine secretions, and gallbladder contraction.
- Glucagon-like peptides are secreted by enteroendocrine L-cells of the ileum and colon and by the nervous system. These peptides decrease gastrointestinal motility and increase proliferation of the gastrointestinal tract; they also influence glucagons and insulin secretion.
- Insulin-like growth factors, also secreted by endocrine cells of the gastrointestinal tract, increase proliferation of the gastrointestinal tract.

Four examples of neurocrine peptides are:

- Vasoactive intestinal polypeptide (VIP) is present in central and peripheral neurons. It is not thought to be present in intestinal endocrine cells. VIP is thought to stimulate intestinal secretions, relax most gastrointestinal sphincters, inhibit gastric acid secretion, and stimulate the release of pancreatic secretions.

- Gastrin-releasing peptide (GRP), also called bombesin, released from enteric nerves, stimulates gastrin release as well as other peptides like cholecystokinin, glucagon-like peptides, and somatostatin.
- Neurotensin, produced by neurons and N-cells of the small intestine mucosa (especially the ileum), has no physiological role in digestion at normal circulating concentrations; however, neurotensin has multiple actions in the brain.
- Substance powder (P), another neuropeptide found in nerve and endocrine cells in the gastrointestinal tract, increases blood flow to the gastrointestinal tract, inhibits acid secretion, increases motility of the small intestine, and binds to pancreatic acinar cells associated with enzyme secretion.

In addition to effects on gastrointestinal tract secretions and motility, many hormones and peptides influence food intake. Ghrelin, for example, a peptide secreted primarily from endocrine cells of the stomach and small intestine, stimulates food intake. Plasma concentrations of ghrelin typically rise before eating (e.g., a fasting situation) and decrease immediately after eating, especially carbohydrates. Ghrelin also stimulates expression of neuropeptide Y. Neuropeptide Y also stimulates eating, but inhibits secretion of gastric ghrelin and insulin. Neuropeptide Y is typically effective as long as leptin concentrations are relatively low.

Leptin is secreted mainly by white adipose tissue and the amount secreted is proportional to fat stores. Leptin suppresses food intake and inhibits neurons from releasing neuropeptide Y and agouti-related protein, with the net effect of suppressing these appetite-stimulating peptides. Leptin is known to work in conjunction with α melanocyte stimulating hormone (α -MSH). Specifically, leptin's ability to inhibit food intake is based, at least in part, on α -MSH's stimulation of MC₄ receptors primarily in the hypothalamus. Corticotropin-releasing factor (CRF) also is involved in leptin's suppression of food intake.

Some of the other hormones that control gastrointestinal tract secretions also have been shown to affect food intake. Cholecystokinin and gastrin-releasing peptides are thought to serve as satiety factors. Thus, the various mediators of the digestive process work in concert to stimulate and inhibit food intake and to break down and absorb the nutrients.

SUMMARY

Examining the various mechanisms in the gastrointestinal tract that allow food to be ingested, digested, and absorbed, and its residue to then be excreted, reveals the complexity of the digestion and absorption processes.

Normal digestion and absorption of nutrients depend not only on a healthy digestive tract but also on integration of the digestive system with the nervous, endocrine, and circulatory systems.

The many factors that influence digestion and absorption—including dispersion and mixing of ingested food, quantity and composition of gastrointestinal secretions, enterocyte integrity, the expanse of intestinal absorptive area, and the transit time of intestinal contents—must be coordinated so that the body can be nourished without disrupting the homeostasis of body fluids. Much of the coordination required is provided by regulatory peptides, some of which are provided by the nervous system as well as by the endocrine cells of the gastrointestinal tract.

Although the basic structure of the digestive tract, which consists of the mucosa, submucosa, muscularis externa, and serosa, remains the same throughout, structural modifications enable various segments of the gastrointestinal tract to perform more specific functions. Gastric glands that underlie the gastric mucosa secrete

fluids and compounds necessary for the stomach's digestive functions. Other particularly noteworthy features are the folds of Kerckring, the villi, and the microvilli, all of which dramatically increase the surface area exposed to the contents of the intestinal lumen. This enlarged surface area helps maximize absorption, not only of ingested nutrients but also of endogenous secretions released into the gastrointestinal tract.

Study of the digestive system makes abundantly clear the fact that a person's adequate nourishment, and therefore his or her health, depends in large measure on a normally functioning gastrointestinal tract. Particularly crucial to nourishment and health is a normally functioning small intestine, because that is where the greatest amount of digestion and absorption occurs. Later chapters of this book expand on digestion and absorption of individual nutrients.

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An Overview of Selected Digestive System Disorders with Implications for Nourishing the Body

In Chapter 2, digestion is defined as a process by which food is broken down mechanically and chemically in the gastrointestinal (GI) tract. Digestion ultimately provides nutrients ready for absorption into the body through the cells of the GI tract, principally the cells of the small intestine (enterocytes). Secretions required to digest nutrients are produced by multiple organs of the GI tract. These secretions include principally enzymes, but also hydrochloric acid important for gastric digestion, and bicarbonate and bile important for digestion and absorption in the intestine. If one or more organs malfunctions because of disease, fewer secretions may be synthesized and released into the GI tract. Without secretions, or with less than normal amounts of secretions, nutrient digestion may be impaired, resulting in nutrient malabsorption.

Many conditions or diseases alter the function of organs of the GI tract and thus affect digestion. For example, some GI tract diseases may cause decreased synthesis and release of secretions needed for nutrient digestion. Other conditions or diseases that affect the GI tract—for example, malfunction of sphincters—can alter motility or clearing of the GI contents through the organs of the GI tract. Clearing problems may cause back fluxes (refluxes) of secretions from, for example, the stomach into the esophagus (remember, normally the contents of the GI tract move from the esophagus to the stomach, and not vice versa). Conditions in which the GI mucosa is inflamed or damaged, as well as conditions that increase transit time or speed up the movement of GI contents (food and nutrients) through the GI tract, typically result in nutrient malabsorption, because the body does not have enough time to digest and absorb nutrients.

An understanding of the physiology of the GI tract and its accessory organs, and of the diseases affecting the GI tract, is essential to understanding how to modify a person's diet from the standard dietary recommendations for healthy populations of the United States. This perspective addresses, in a general fashion, four disorders that affect the gastrointestinal tract and outlines the implications of these conditions for nourishing the body.

Disorder 1: Gastroesophageal Reflux Disease

Gastroesophageal reflux disease (GERD) is a disorder marked by reflux or backward flow of gastric contents (acidic chyme) from the stomach to the esophagus.

After food is chewed and swallowed, the food enters the esophagus and then passes through the gastroesophageal sphincter into the stomach. Normally, the gastroesophageal sphincter displays a relatively high pressure that prevents the reflux of stomach contents into the esophagus. However, changes or decreases in the gastroesophageal sphincter pressure, sometimes called lower esophageal sphincter incompetence, can ultimately result in GERD. Increases in abdominal pressure, such as may occur with overeating, bending, lifting, lying down, vomiting, or coughing, also can increase reflux and cause GERD.

Recurrent reflux of gastric contents, including hydrochloric acid, into the esophagus from the stomach can damage and inflame the esophageal mucosa and result in reflux esophagitis (inflammation of the esophagus caused by the refluxed gastric contents). The severity of the esophagitis depends in part on the volume and acidity of the gastric contents that are refluxed and on the length of time the gastric contents are in contact with the esophageal mucosa. The more acidic the contents, and the longer the contents are in contact with the mucosa, the more damage results. Weak peristalsis and delays in gastric emptying are likely to prolong contact time and increase damage. The resistance of the esophageal mucosa also affects the severity of the damage. Repeated bouts of GERD resulting in reflux esophagitis cause, to varying degrees, esophageal edema (swelling); esophageal tissue damage, including erosion and ulceration; blood vessel (usually capillary) damage; spasms; and fibrotic tissue formation, which can cause a narrowing (stricture) within the esophagus.

A person experiencing GERD or reflux esophagitis typically complains of heartburn, that is, a burning sensation in the midchest region. The symptoms usually occur within an hour of eating and worsen if the person lies down soon after eating.

To address nutrition implications of this condition, we first need to reexamine some of the foods, nutrients, or substances in foods that influence gastroesophageal sphincter pressure, that may promote increased acid production, and that may irritate an inflamed esophagus. Several substances decrease gastroesophageal sphincter pressure, including high-fat foods, chocolate, nicotine, alcohol, and carminatives [1–5]. Carminatives are volatile oil extracts of plants, most often oils of spearmint and peppermint. Other substances increase gastric secretions, especially acid production. Alcohol, calcium, decaffeinated

and caffeinated coffee, and tea (specifically, methylxanthines) stimulate gastric secretions, including hydrochloric acid [6–10]. Citrus products and other acidic foods or beverages, as well as some spices, are known to directly irritate an inflamed esophagus. Ingesting these substances or foods is likely to aggravate irritated esophageal mucosa.

Based on this knowledge, some of the recommendations for the patient with GERD or reflux esophagitis have the specific goal of avoiding:

- substances that can further decrease lower gastroesophageal sphincter pressure, which is already low because of the condition
- substances that may promote the secretion of acid, which would be present in higher concentrations than normal if refluxed
- foods or substances that may irritate an inflamed esophagus

To implement these recommendations, people with GERD or reflux esophagitis must be told which foods or substances to avoid, such as high-fat foods or meals, chocolate, coffee, tea, alcohol, carminatives such as peppermint and spearmint, citrus products, acidic foods, and spices such as red and black pepper, nutmeg, cloves, and chili powder.

In addition to avoiding substances that reduce gastroesophageal sphincter pressure, that promote the secretion of acid, and that can irritate an inflamed esophagus, recommendations can also include increasing the intake of foods or nutrients that increase gastroesophageal sphincter pressure. Protein is a nutrient that increases gastroesophageal sphincter pressure [10]. Consequently, a higher than normal protein intake is encouraged; however, excessive protein intakes, especially from foods high in calcium such as dairy products, are not recommended. The reason for avoiding excessively high intakes of dairy foods relates to the fact that the amino acids and peptides (generated by digesting the protein in the dairy products) and calcium in dairy products are known to stimulate gastrin release [11]. Although gastrin increases gastroesophageal sphincter pressure, it is also a potent stimulator of hydrochloric acid secretion.

In addition to noting the previously stated nutrition recommendations, remember that reflux is more likely to occur with increased gastric volume (i.e., eating large meals), increased gastric pressure (e.g., from obesity), and placement of gastric contents near the sphincter (i.e., bending, lying down, or assuming a recumbent

position). Thus, recommendations for people with GERD or reflux esophagitis should include these measures:

- eating smaller meals (and avoiding large ones)
- drinking fluids between meals, instead of with a meal, to help minimize large increases in gastric volume
- losing weight, if the person is overweight or obese
- avoiding tight-fitting clothes
- not avoiding lying down, lifting, or bending for at least 2 hours after eating

Disorder 2: Inflammatory Bowel Diseases

Inflammatory bowel diseases (IBDs) include ulcerative colitis and Crohn's disease (also called regional enteritis) and are characterized by acute, relapsing, or chronic inflammation of various segments of the GI tract, especially the intestines. Although the causes of IBDs are unclear, nutrient malabsorption is a significant problem for several reasons. First, because of the disease-associated inflammation of the mucosa, brush border disaccharidase and peptidase activities are diminished, and thus nutrient digestion is impaired. Second, nutrient transit time is typically decreased—that is, GI tract contents move through the GI tract more quickly than usual and thus leave little time for absorption. Third, malabsorption occurs because of direct damage to the absorptive mucosa cells. Exacerbating the poor nutrient absorption is poor food intake, which is especially common during acute attacks.

Manifestations of IBDs include excessive diarrhea and steatorrhea (large amounts of fat in the feces), which may occur up to 20 times per day. Diarrhea is associated with increased losses of fluid and electrolytes (especially potassium) from the body. Fluid and electrolyte imbalance or even dehydration can result. Blood is often present in the feces, especially if deeper areas of the GI mucosa are severely inflamed or ulcerated. Loss of blood impairs the body's protein and mineral (especially iron) status. If IBD has affected the ileum (as is common with Crohn's disease), vitamin B₁₂ absorption may be impaired

(this vitamin is absorbed in the ileum), reabsorption of bile salts from the ileum may be diminished, and fat malabsorption may occur. Although pancreatic lipase is available to hydrolyze dietary triglycerides, the lack of sufficient bile or diminished bile function caused by bacterial alteration of bile can decrease micelle formation and thus decrease absorption of fatty acids and fat-soluble vitamins into the enterocyte. Unabsorbed fatty acids bind to calcium and magnesium in the lumen of the intestine; the resulting insoluble complex, sometimes called a soap, is excreted in the feces.

Dietary recommendations for people with IBD are aimed at replacing nutrient losses, correcting nutrient imbalances, and improving nutrition status. Some of these dietary recommendations include:

- increasing iron intake above the RDA, because of increased iron losses with the bloody diarrhea and decreased absorption
- following a low-fat diet, because fat absorption is impaired
- increasing calcium and magnesium intake, because absorption of these nutrients is diminished by soap formation and overall malabsorption with diarrhea
- getting a high-protein diet, because protein is lost from the blood into the feces with bloody diarrhea and malabsorption of amino acids
- taking fat-soluble vitamin supplements, possibly in a water-miscible form to improve absorption
- increasing fluid and electrolyte intake to rehydrate and restore electrolyte balance
- increasing overall intake of nutrients to meet energy and nutrient needs

Easily digestible carbohydrate-rich foods that are low in fiber, high-protein low-fat foods with minimal residue, and lactose-free foods should provide the bulk of the person's energy needs if oral intake is deemed appropriate. Medium-chain triacylglycerol (MCT) oil, which is absorbed directly into portal blood and does not need bile for absorption, may be added in small amounts to different foods throughout the day to

increase energy intake. Sometimes, however, complete rest of the GI tract is needed, and a person with IBD may need to be fed intravenously (by parenteral nutrition).

Disorder 3: Celiac Disease

Celiac disease, also called gluten- or gliadin-sensitive enteropathy or celiac sprue, results from an intolerance to gluten. Gluten is the general name for storage proteins, also called prolamins, in grains. Grains vary in their storage proteins, however, and in people with celiac disease, three storage proteins—secalin in rye, hordein in barley, and gliadin in wheat—appear to elicit or trigger the problems. In addition, the storage protein avenin, in oats, may also be problematic, especially for younger people with the condition.

Consuming any of these grains alone, or foods made with any of these grains, triggers both immune and inflammatory responses in a person with celiac disease. Although the severity of the condition varies, the small intestine of someone with celiac disease becomes inflamed; lymphocytes and other immune system cells and the cytokines produced by the cells invade and attack the mucosa. The villi typically become atrophied or blunted, with corresponding changes in the crypt to villous height ratio. Because of the villi destruction, digestion and absorption become severely impaired. Manifestations of celiac disease include diarrhea, abdominal pain, malabsorption, and weight loss. Over time and if untreated, an infant or child with celiac disease may even exhibit signs of protein-energy malnutrition characterized by poor somatic muscle mass, hypotonia, abdominal distension, peripheral edema, depleted subcutaneous fat stores, and poor growth. Older children also may complain of constipation, nausea, reflux, and vomiting. This disorder affects other parts of the body as well as the intestines. Extraintestinal symptoms often include skin rashes, and muscle and joint pain. Fertility problems, especially in women with celiac disease, have been noted, along with bone problems including delayed bone growth and development and ultimately osteoporosis.

The cause of celiac disease is not clear, but it is thought to have a genetic component. The condition has been linked to presence of several specific human

leukocyte antigens. Diagnosis of celiac disease is based on the presence of a combination of serum antibody markers and biopsy of the small intestine.

Treatment of celiac disease requires lifelong exclusion of any form of any product that contains rye, barley, or wheat. However, because many foods contain combinations of grains, the list of foods to exclude is quite extensive. For example, grains such as triticale, which is a combination of rye and wheat, and malt, which is a partial hydrolysate of barley, cannot be consumed. Because oats are often harvested and milled with wheat, a risk of contamination exists, and oats are therefore usually excluded too from the celiac diet. A list of all the foods allowed and not allowed with celiac disease is beyond the scope of this perspective. Research is often required before a food can be safely consumed. For example, starch products are usually made from cornstarch and would be allowed, but modified food starch can be made from either corn or wheat, so the person would need to contact the manufacturer. Similarly, dextrin, a common ingredient in foods, can be made from corn, potato, arrowroot, rice, tapioca, or wheat, and thus the manufacturer would have to be contacted to verify the exact source of the dextrin. Food manufacturers do not need to list on the label whether a food is gluten free, so the person with celiac disease and health professionals must do the research.

Disorder 4: Chronic Pancreatitis

Pancreatitis, or inflammation of the pancreas, provides an excellent example of the nutritional ramifications of a condition affecting an accessory organ of the GI tract. Remember that the exocrine portion of the pancreas produces several enzymes needed to digest all nutrients. **Chronic** refers to an ongoing or long-lasting situation.

Chronic pancreatitis can result from long-time excessive use of alcohol, gallstones, liver disease, viral infections, and use of certain medications, among other factors. With time, sections of pancreatic tissue become dysfunctional. Acinar cells, for example, can ultimately fail to produce sufficient digestive enzymes and juices. Consequently, a person with the chronic pancreatitis experiences severe pain, especially with eating, as well as nausea, vomiting, and diarrhea. The diarrhea

results in part from the maldigestion, with resulting malabsorption of several nutrients.

Diminished secretion of pancreatic lipase into the duodenum, caused by the chronic pancreatitis, results in maldigestion of fat and thus malabsorption of fat and fat-soluble vitamins. Fat is malabsorbed because not enough pancreatic lipase is available to hydrolyze the fatty acids from the triglycerides. This hydrolysis is necessary in order for fatty acids and monoacylglycerols to form micelles, the form in which the fatty acids are carried into the enterocyte for absorption. Thus, with pancreatitis, the insufficiency of enzymes available for fat hydrolysis necessitates a low-fat diet.

In addition to insufficient pancreatic lipase secretion, bicarbonate secretion into the duodenum is also diminished with pancreatitis. Bicarbonate, in part, increases the pH of the small intestine. Intestinal enzymes function best at an alkaline pH, which is provided by the release of bicarbonate into the intestine. Oral supplements of pancreatic enzymes may be needed to replace the diminished output of these enzymes by the malfunctioning, inflamed pancreas. Medications such as antacids, H₂ receptor blockers, or proton pump inhibitors may also be needed. The medications are taken to diminish acid production and thus increase intestinal pH. In effect, they replace the bicarbonate and thus help maintain an appropriate pH for enzyme function. Exogenous insulin may also have to be administered if insulin is no longer produced in sufficient quantities by the damaged pancreatic endocrine cells.

These four conditions illustrate how diseases that affect the GI tract—malfunction of a sphincter (GERD and reflux esophagitis), destruction of enterocyte function (IBD), destruction of the enterocyte absorptive surface (celiac disease), and chronic malfunction of a GI tract accessory organ that provides secretions needed for nutrient digestion (pancreatitis)—affect the body's ability to digest and absorb nutrients. Furthermore, these conditions illustrate how nutrient intakes must deviate from recommended levels—in some cases to lower levels, and in other cases to higher levels—depending on the condition. Such dietary modifications are typical of many conditions that affect not only the gastrointestinal tract but also other organ systems.

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3

Carbohydrates

Structural Features

Simple Carbohydrates

Monosaccharides
Disaccharides

Complex Carbohydrates

Oligosaccharides
Polysaccharides

Digestion

Digestion of Polysaccharides
Digestion of Disaccharides

Absorption, Transport, and Distribution

Absorption of Glucose and Galactose
Absorption of Fructose
Monosaccharide Transport and Cellular Uptake
Glucose Transporters
Insulin
Maintenance of Blood Glucose Levels

Glycemic Response to Carbohydrates

Glycemic Index
Glycemic Load

Integrated Metabolism in Tissues

Glycogenesis
Glycogenolysis
Glycolysis
Substrate-Level Phosphorylation
The Tricarboxylic Acid Cycle
Formation of ATP
The Hexosemonophosphate Shunt (Pentose Phosphate Pathway)
Gluconeogenesis

Regulation of Metabolism

Allosteric Enzyme Modulation
Regulatory Effect of NADH:NAD⁺ Ratio
Hormonal Regulation
Directional Shifts in Reversible Reactions

PERSPECTIVE

Hypoglycemia: Fact or Fall Guy?

The major source of energy fuel in the average human diet is carbohydrate, supplying half or more of the total caloric intake. Roughly half of dietary carbohydrate is in the form of polysaccharides such as starches and dextrans, derived largely from cereal grains and vegetables. The remaining half is supplied as simple sugars, the most important of which include sucrose, lactose, and, to a lesser extent, maltose, glucose, and fructose.

Structural Features

Carbohydrates are polyhydroxy aldehydes or ketones, or substances that produce these compounds when hydrolyzed. They are constructed from the atoms of carbon, oxygen, and hydrogen. These atoms occur in a proportion that approximates that of a “hydrate of carbon,” CH₂O, accounting for the term *carbohydrate*. Carbohydrates comprise two major classes: simple carbohydrates and complex carbohydrates. Simple carbohydrates include monosaccharides and disaccharides. Complex carbohydrates include oligosaccharides, containing 3 to 10 saccharide units and the polysaccharides contain more than 10 units (Figure 3.1).

Simple Carbohydrates

- **Monosaccharides** are structurally the simplest form of carbohydrate in that they cannot be reduced in size to smaller carbohydrate units by hydrolysis. Monosaccharides are called simple sugars and are sometimes referred to as monosaccharide units. The most abundant monosaccharide in nature—and certainly the most important nutritionally—is the 6-carbon sugar glucose.
- **Disaccharides** consist of two monosaccharide units joined by covalent bonds. Within this group, sucrose, consisting of one glucose and one fructose residue, is nutritionally the most significant, furnishing approximately one-third of total dietary carbohydrate in an average diet.

Complex Carbohydrates

- **Oligosaccharides** consist of short chains of monosaccharide units that are also joined by covalent bonds. The number of units is designated by the prefixes *tri-*, *tetra-*, *penta-*, and so on, followed by the word *saccharide*. Among the oligosaccharides, trisaccharides occur most frequently in nature.
- **Polysaccharides** are long chains of monosaccharide units that may number from several into the hundreds or even thousands. The major polysaccharides of interest in nutrition are glycogen, found in certain animal tissues, and starch and cellulose, both of plant origin. All these polysaccharides consist of only glucose units.

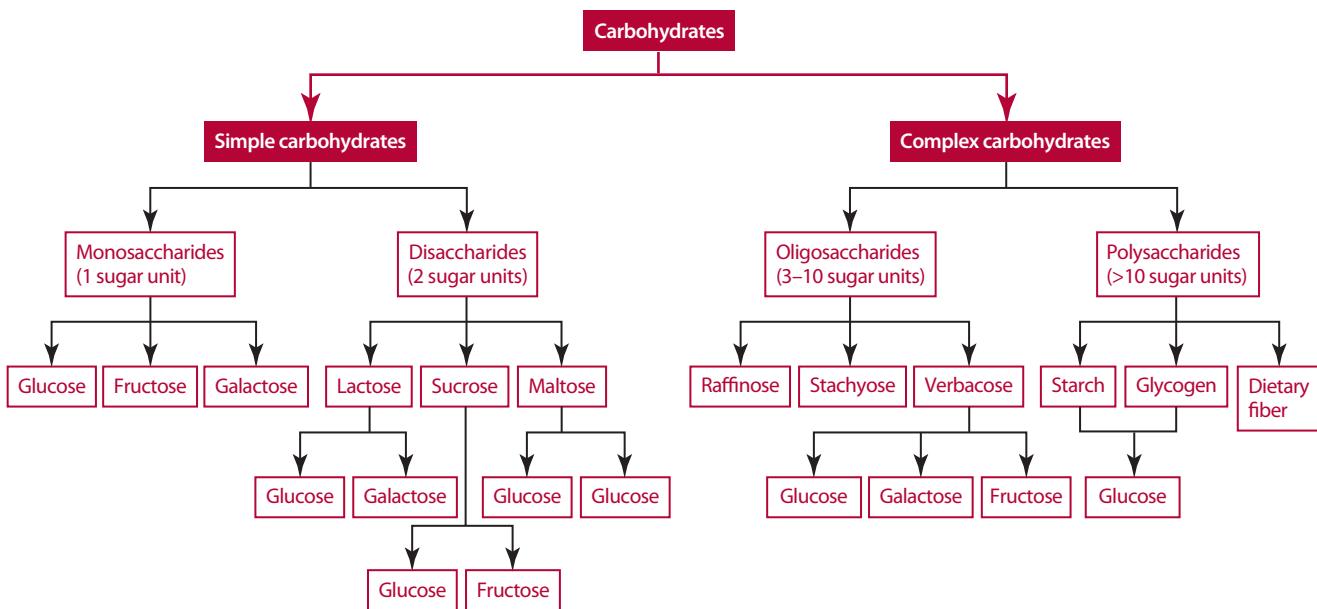


Figure 3.1 Classification of carbohydrates.

Simple Carbohydrates

MONOSACCHARIDES

As monosaccharides occur in nature or arise as intermediate products in digestion, they contain from three to seven carbon atoms and accordingly are termed trioses, tetroses, pentoses, hexoses, and heptoses. They cannot be further broken down with mild conditions. In addition to hydroxyl groups, these compounds possess a functional carbonyl group, C=O, that can be either an aldehyde or a ketone, leading to the additional classification of aldoses, sugars having an aldehyde group, and ketoses, sugars possessing a ketone group. These two classifications together with the number of carbon atoms describe a particular monosaccharide. For example, a five-carbon sugar having a ketone group is a ketopentose; a six-carbon aldehyde-possessing sugar is an aldohexose, and so forth.

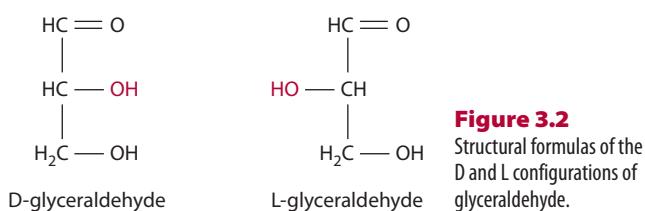
Stereoisomerism

A brief discussion of stereoisomerism as it relates to carbohydrates is provided here because most biological systems are stereospecific. (For a more extensive discussion refer to a general biochemistry text [1]). Many organic substances, including carbohydrates, are optically active: If plane-polarized light is passed through a solution of the substances, the plane of light is rotated to the right (for dextrorotatory substances) or to the left (for levorotatory ones). The direction and extent of the rotation are characteristic of a particular compound and depend on the substance's concentration and temperature and the wavelength of the light. The right or left direction of light rotation is expressed as + (dextrorotatory) or - (levorotatory),

and the number of angular degrees indicates the extent of rotation.

Optical activity is attributed to the presence of one or more asymmetrical or chiral carbon atoms in the molecule. **Chiral carbon** atoms have four different atoms or groups covalently attached to them. Aldoses with at least three carbon atoms and ketoses with at least four carbons have a chiral carbon atom. Because different groups are attached, it is not possible to move any two atoms or groups to other positions and rotate the new structure so it can be superimposed on the original. The moving of groups instead creates a pair of molecules that are mirror images of each other. The molecules are said to be enantiomers, a special class within a broader family of compounds called **stereoisomers**. Stereoisomers are compounds having two or more chiral carbon atoms that have the same four groups attached to those carbon atoms but are not mirror images of each other.

If an asymmetrical substance rotates the plane of polarized light a certain number of degrees to the right, its enantiomer rotates the light the same number of degrees to the left. Enantiomers exist in D or L orientation, and if a compound is structurally D, its enantiomer is L. The D and L designation does not predict the direction of rotation of plane-polarized light. Instead, the designation is simply a structural analogy to the reference compound glyceraldehyde. Its D and L forms are, by convention, drawn as shown in Figure 3.2. The D and L designation originally was assigned arbitrarily, but it was later proven that the structure designated as the D form rotates light to the right (dextrorotatory). For carbohydrates the distinction between D and L configurations of enantiomers therefore rests with the direction of the —OH bond on the single chiral carbon of the molecule. Note that in the D configuration the —OH on the chiral carbon points to the right



and in the L configuration to the left. Remember, these forms are not superimposable.

Monosaccharides with more than three carbons have more than one chiral center. In such cases, the highest-numbered chiral carbon indicates whether the molecule is of the D or the L configuration. Monosaccharides of the D configuration are much more important nutritionally than their L isomers, because D isomers exist as such in dietary carbohydrate and are metabolized specifically in that form. The reason for this specificity is that the enzymes involved in carbohydrate digestion and metabolism are stereospecific for D sugars, meaning that they react only with D sugars and are inactive toward L forms. The D and L forms of glucose and fructose are shown in Figure 3.3. Note that all of the —OH groups of the stereoisomers are flipped to the opposite side.

In Figure 3.3 the structures of glucose and fructose are shown as open-chain models, in which the carbonyl (aldehyde or ketone) functions are free. The monosaccharides generally do not exist in open-chain form, as explained later, but they are shown that way here to clarify the D-L concept and to illustrate the so-called **anomeric carbon**, the carbon atom comprising the carbonyl function. Notice that the anomeric carbon is number 1 in the aldose (glucose) and number 2 in the ketose (fructose).

Ring Structures

In solution, the monosaccharides do not exist in an open-chain form. They do not undergo reactions characteristic of

true aldehydes and ketones. Instead, the molecules cyclize by a reaction between the carbonyl group and a hydroxyl group. If the sugar contained an aldehyde, it is called a hemiacetal; if the sugar contained a keto group, it is called a hemiketal. This formation of the cyclic structures forms an additional chiral carbon. Therefore, the participating groups within a monosaccharide are the aldehyde or ketone of the anomeric carbon atom and the alcohol group attached to the highest numbered chiral carbon atom, as illustrated in Table 3.1 using the examples of D-glucose, D-galactose, and D-fructose. The formation of the hemiacetal or hemiketal produces a new chiral center at the anomeric carbon, designated by an asterisk in the structures in Table 3.1, and therefore the bond direction of the newly formed hydroxyl becomes significant. In the cyclized structures shown (known as Fisher projections), the anomeric hydroxyls are arbitrarily positioned to the right, resulting in an alpha (α) configuration. If the anomeric hydroxyl were directed to the left, the structure would be in a beta (β) configuration. Cyclization to the hemiacetal or hemiketal can produce either the α - or the β -isomer. In aqueous solution, an equilibrium mixture of the α , β , and open isomers exists, with the concentration of the β form roughly twice that of the α form. In essence, the α -hemiacetal can change to the open structure and again form a ring, with either the α or the β configuration.

Stereoisomerism among the monosaccharides, and also among other nutrients such as amino acids and lipids, has important metabolic implications because of the stereospecificity of certain metabolic enzymes. An interesting example of stereospecificity is the action of the digestive enzyme α -amylase, which hydrolyzes polyglucose molecules such as starches, in which the glucose units are connected through an α -linkage. Cellulose is also a polymer of glucose, but one in which the monomeric glucose residues are connected by β -linkages, and it is resistant to α -amylase hydrolysis present in the human digestive system.

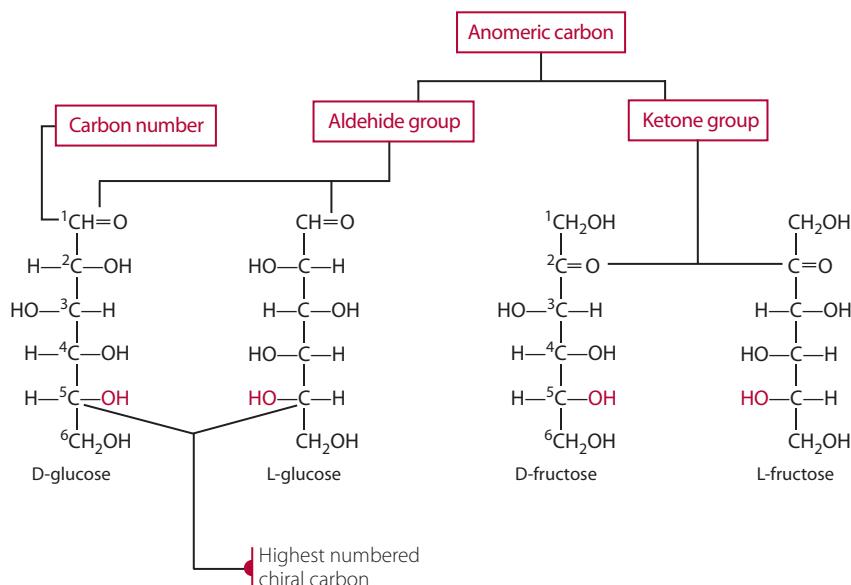


Figure 3.3 Structural (open-chain) models of the D and L forms of the monosaccharides glucose and fructose.

Haworth Models

The structures of the cyclized monosaccharides are more conveniently and accurately represented by Haworth models. In such models the carbons and oxygen comprising the five- or six-membered ring are depicted as lying in a horizontal plane, with the hydroxyl groups pointing down or up from the plane. Those groups directed to the right in the open-chain structure point down in the Haworth model, and those directed to the left point up. Table 3.1 shows the structural relationship of simple projection and Haworth formulas for the major naturally occurring hexoses: glucose, galactose, and fructose. Remember that in solution the cyclic monosaccharides open and close to form an equilibrium between the α and the β forms. Regardless of how the cyclic structure is written, the molecule exists in both forms in solution unless the anomeric carbon has formed a chemical bond and is no longer able to open and close. The different ways of drawing the structures are presented here because all are used in the nutrition literature. Chemists often portray the structures to show the true bond angles. The structures can be shown in a boat configuration

or a chair configuration. Additional information can be obtained from a biochemistry textbook [1].

Pentoses

Compared to the hexoses, pentose sugars furnish very little dietary energy because relatively few are available in the diet. However, they are readily synthesized in the cell from hexose precursors and are incorporated into metabolically important compounds. The aldopentose ribose, for example, is a constituent of key nucleotides such as the adenosine phosphates—adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), cyclic adenosine monophosphate (cAMP), and the nicotinamide adenine dinucleotides (NAD^+ , NADP^+). Ribose and its deoxygenated form, deoxyribose, are part of the structures of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), respectively. Ribitol, a reduction product of ribose, is a constituent of the vitamin riboflavin and of the flavin coenzymes; flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). The structural formulas of ribose, deoxyribose, and ribitol are depicted in Figure 3.4.

Table 3.1 Various Structural Representations Among the Hexoses: Glucose, Galactose, and Fructose

Hexose	Fisher Projection	Cyclized Fisher Projection	Haworth	Simplified Haworth
α -D-glucose				
β -D-galactose				
β -D-fructose				

*The anomeric carbon.

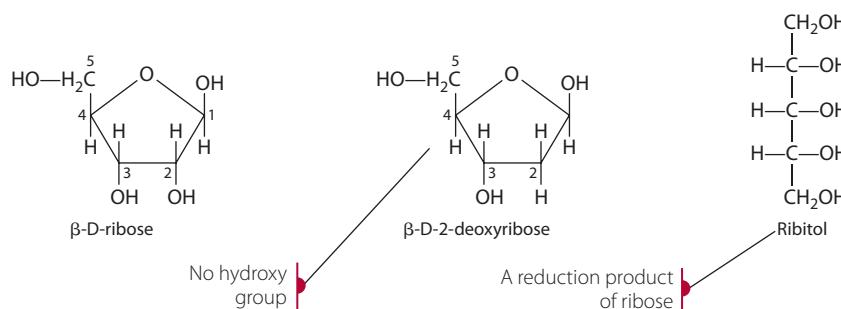


Figure 3.4 Structural formulas of the pentoses ribose and deoxyribose and of the alcohol ribitol.

Reducing Sugars

Monosaccharides that are cyclized into hemiacetals or hemiketals are sometimes called reducing sugars because they are capable of reducing other substances, such as the copper ion from Cu^{2+} to Cu^+ . This property is useful in identifying which end of a polysaccharide chain has the monosaccharide unit that can open and close. This role of reducing sugars is discussed in more detail in the section on polysaccharides.

DISACCHARIDES

Disaccharides contain two monosaccharide units attached to one another through acetal bonds. Acetal bonds, also called glycosidic bonds because they occur in the special case of carbohydrate structures, are formed between a hydroxyl group of one monosaccharide unit and a hydroxyl group of the next unit in the polymer, with the elimination of one molecule of water. The glycosidic bonds generally involve the hydroxyl group on the anomeric carbon of one member of the pair of monosaccharides and the hydroxyl group on carbon 4 or 6 of the second member. Furthermore, the glycosidic bond can be α or β in orientation, depending on whether the anomeric hydroxyl group was α or β before the glycosidic bond was formed and on the specificity of the enzymatic reaction catalyzing their formation. Specific

glycosidic bonds therefore may be designated α 1-4, β 1-4, α 1-6, and so on. Disaccharides are major energy-supplying nutrients in the diet. The most common disaccharides are maltose, lactose, and sucrose (Figure 3.5).

Maltose Maltose is formed primarily from the partial hydrolysis of starch and therefore is found in malt beverages such as beer and malt liquors. It consists of two glucose units linked through an α 1-4 glycosidic bond. The structure on the right in Figure 3.5 is shown in the β form, although it also may exist in the α form.

Lactose Lactose is found naturally only in milk and milk products. It is composed of galactose linked by a β 1-4 glycosidic bond to glucose. The glucose can exist in either α or β form (Figure 3.5).

Sucrose Sucrose (cane sugar, beet sugar) is the most widely distributed of the disaccharides and is the most commonly used natural sweetener. It is composed of glucose and fructose and is structurally unique in that its glycosidic bond involves the anomeric hydroxyl of both residues. The linkage is α with respect to the glucose residue and β with respect to the fructose residue (Figure 3.5). Because it has no free hemiacetal or hemiketal function, sucrose is not a reducing sugar.

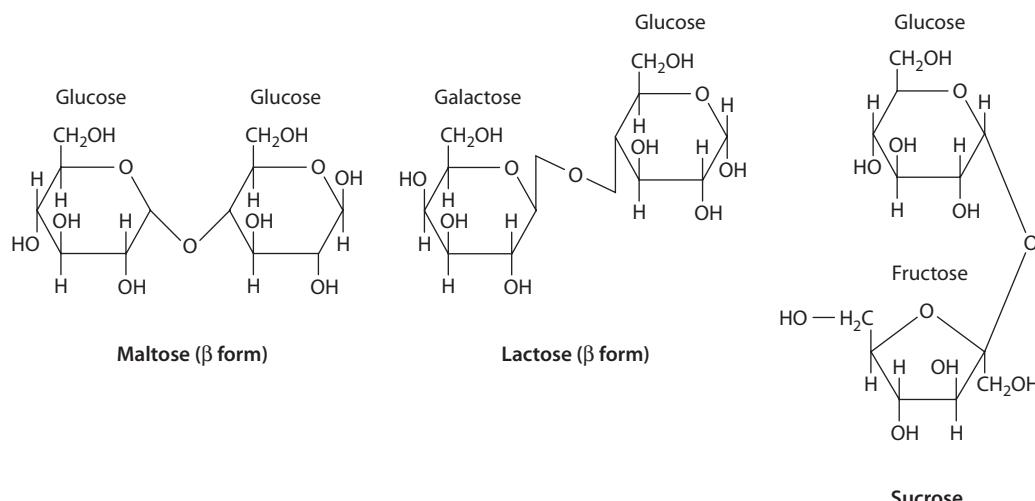


Figure 3.5 Common disaccharides.

Complex Carbohydrates

OLIGOSACCHARIDES

Raffinose (a trisaccharide), **stachyoses** (a tetrasaccharide), and **verbascose** (a pentasaccharide) are made up of glucose, galactose, and fructose and are found in beans, peas, bran, and whole grains. Human digestive enzymes do not hydrolyze them, but the bacteria within the intestine can digest them. This is the basis for flatulence that occurs after eating these foods.

POLYSACCHARIDES

The glycosidic bonding of monosaccharide residues may be repeated many times to form high-molecular-weight polymers called polysaccharides. If the structure is composed of a single type of monomeric unit, it is called a homopolysaccharide. If two or more different types of monosaccharides make up its structure, it is called a heteropolysaccharide. Both types exist in nature; however, homopolysaccharides are of far greater importance in nutrition because of their abundance in many natural foods. The polyglucoses starch and glycogen, for example, are the major storage forms of carbohydrate in plant and animal tissues, respectively. Polyglucoses range in molecular weight from a few thousand to 500,000.

The reducing property of a saccharide is useful in describing polysaccharide structure by enabling one end of a linear polysaccharide to be distinguished from the other. In a polyglucose chain, for example, the glucose residue at one end of the chain has a hemiacetal group because its anomeric carbon atom is not involved in acetal bonding to another glucose residue. The residue at the other end of the chain is not in hemiacetal form because it is attached by acetal bonding to the next residue in the chain. A linear polyglucose molecule therefore has a reducing end (the hemiacetal end) and a nonreducing end (at which no hemiacetal exists). This notation is useful in designating at which end of a polysaccharide certain enzymatic reactions occur.

Starch

The most common digestible polysaccharide in plants is starch. It can exist in two forms, amylose and amylopectin, both of which are polymers of D-glucose. The amylose molecule is a linear, unbranched chain in which the glucose residues are attached solely through α 1-4 glycosidic bonds. In water, amylose chains adopt a helical conformation shown in Figure 3.6a. Amylopectin, on the other hand, is a branched-chain polymer, with branch points occurring through α 1-6 bonds, as illustrated in Figure 3.6b. Both amylose and amylopectin occur in cereal grains, potatoes, legumes, and other vegetables. Amylose contributes

about 15% to 20%, and amylopectin 80% to 85%, of the total starch content.

Glycogen

The major form of stored carbohydrate in animal tissues is glycogen, which is localized primarily in liver and skeletal muscle. Like amylopectin, it is a highly branched polyglucose molecule. It differs from the starch only in the fact that it is more highly branched (Figure 3.6c). The glucose residues within glycogen serve as a readily available source of glucose. When dictated by the body's energy demands, glucose residues are sequentially removed enzymatically from the nonreducing ends of the glycogen chains and enter energy-releasing pathways of metabolism. This process, called **glycogenolysis**, is discussed later in this chapter. The high degree of branching in glycogen and amylopectin offers a distinct metabolic advantage, because it presents a large number of nonreducing ends from which glucose residues can be cleaved.

Cellulose

Cellulose is the major component of cell walls in plants. Like the starches, it is a homopolysaccharide of glucose. It differs from the starches in that the glycosidic bonds connecting the residues are β 1-4, rendering the molecule resistant to the digestive enzyme α -amylase, which is stereospecific to favor α 1-4 linkages. Because cellulose is not digestible by mammalian digestive enzymes, it is defined as a dietary fiber and is considered not to provide energy. However, as a source of fiber, cellulose assumes importance as a bulking agent and a potential energy source for some intestinal bacteria that are able to digest it. The extent to which the energy is available for absorption in the human is debated. A very limited amount likely is available in the form of short-chain fatty acids.

Digestion

The most important dietary carbohydrates nutritionally are polysaccharides and disaccharides, because free monosaccharides are not commonly present in the diet in significant quantities. However, some free glucose and fructose are present in honey, certain fruits, and the carbohydrates added to processed foods such as high-fructose corn syrup. The cellular use of carbohydrates depends on their absorption from the gastrointestinal (GI) tract into the bloodstream, a process normally restricted to monosaccharides. Polysaccharides and disaccharides therefore must be hydrolyzed to their constituent monosaccharide units. The hydrolytic enzymes involved are collectively called **glycosidases** or, alternatively, **carbohydrases**.

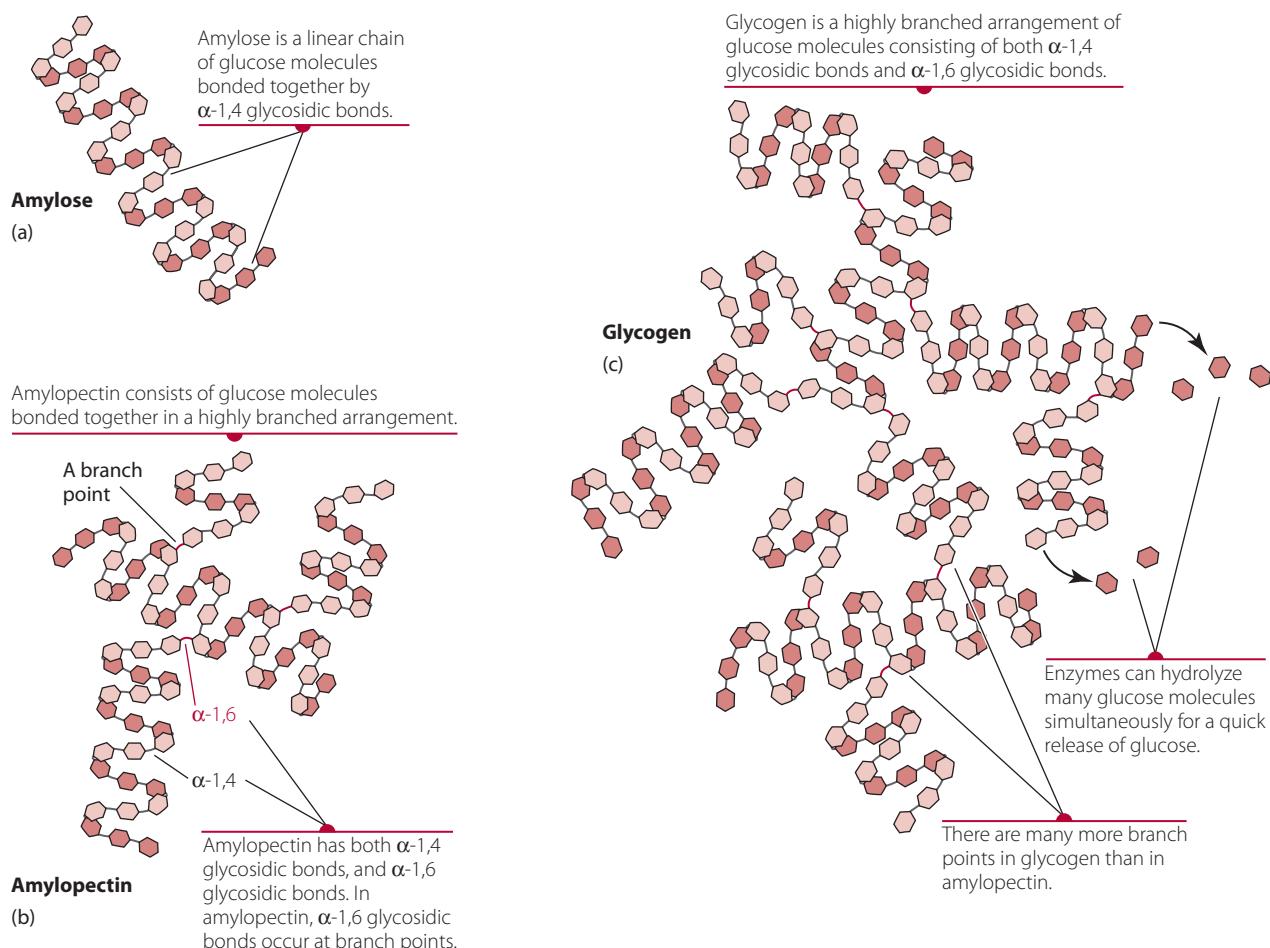


Figure 3.6 Structure of starches and glycogen.

DIGESTION OF POLYSACCHARIDES

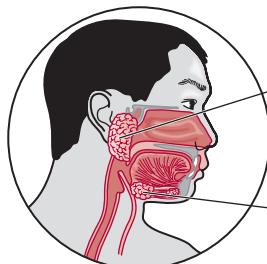
The digestion of polysaccharides starts in the mouth. The key enzyme is salivary α -amylase, a glycosidase that specifically hydrolyzes α 1-4 glycosidic linkages. The β 1-4 bonds of cellulose, the β 1-4 bonds of lactose, and the α 1-6 linkages that form branch points in starch amylopectin are resistant to the action of this enzyme. Given the short period of time that food is in the mouth before being swallowed, this phase of digestion produces few monosaccharides. However, the salivary amylase action continues in the stomach until the gastric acid penetrates the food bolus and lowers the pH sufficiently to inactivate the enzyme. By this point, the starches have been partially hydrolyzed, with the major products being dextrins, which are short-chain polysaccharides and maltose (Figure 3.7). Further digestion of the dextrins is resumed in the small intestine by the α -amylase of pancreatic origin, which is secreted into the duodenal contents. The presence of pancreatic bicarbonate in the duodenum elevates the pH to a level favorable for enzymatic function. If the dietary starch form is amylose, which is unbranched, the products of α -amylase hydrolysis are maltose and the trisaccharide

maltotriose, which undergoes slower hydrolysis to maltose and glucose. The hydrolytic action of α -amylase on amylopectin, a branched starch, produces glucose and maltose, as it does with amylose. However, the α 1-6 bonds linking the glucose residues at the branch points of the molecule cannot be hydrolyzed by α -amylase. Consequently, disaccharide units called isomaltose, possessing α 1-6 glycosidic bonds, are released.

In summary, the action of α -amylase on dietary starch releases maltose, isomaltose, and glucose as principal hydrolytic products, as illustrated in Figure 3.7. The further breakdown of the disaccharide products to glucose is brought about by specific glycosidases described in the section on disaccharides below.

Resistant Starches

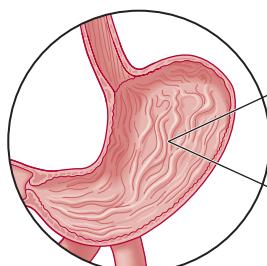
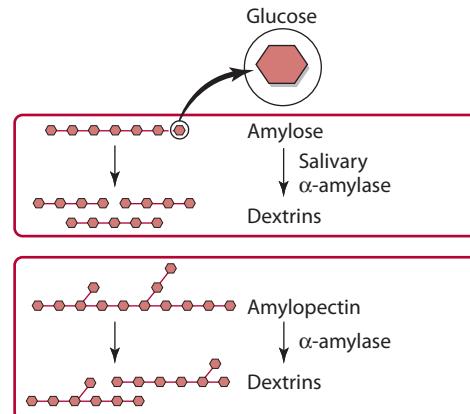
Crystalline starch is insoluble in water and is nondigestible. Crystalline starch is gelatinized by heating (such as in cooking), and in this form it is digestible. On cooling, some starch reverts back to the crystalline form and again becomes resistant to digestion. Starches also can be chemically modified to make them more resistant to digestion,



A. Digestion of amylose and amylopectin in the mouth

Amylose: Salivary glands release salivary α -amylase, which hydrolyzes α -1,4 glycosidic bonds in amylose, forming dextrins.

Amylopectin: Salivary glands release salivary α -amylase, which hydrolyzes α -1,4 glycosidic bonds in amylopectin, forming dextrins.



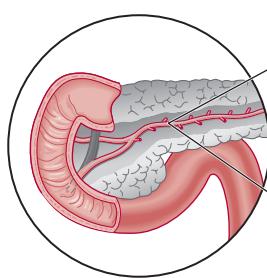
B. There is no digestion of amylose and amylopectin in the stomach

Amylose: Acidity of gastric juice destroys the enzymatic activity of α -amylase. The dextrins pass unchanged into the small intestine.

Amylopectin: Acidity of gastric juice destroys the enzymatic activity of salivary α -amylase. The dextrins pass unchanged into the small intestine.

No further digestion

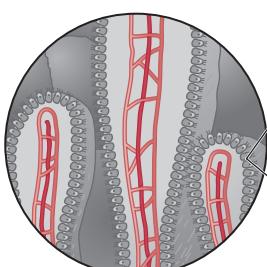
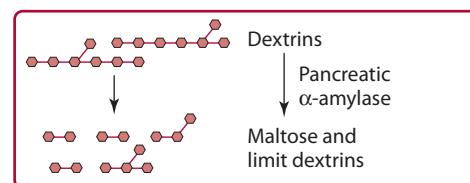
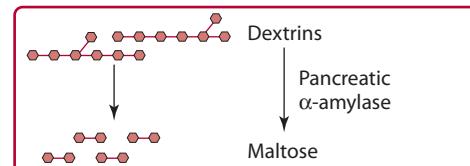
No further digestion



C. Digestion of amylose and amylopectin in the small intestine

Amylose: The pancreas releases pancreatic α -amylase into the small intestine which hydrolyzes α -1,4 glycosidic bonds. Dextrins are broken down into maltose.

Amylopectin: The pancreas releases pancreatic α -amylase into the small intestine, which hydrolyzes α -1,4 glycosidic bonds. Dextrins are broken down into maltose and limit dextrins.



D. Digestion of amylose and amylopectin on the brush border of the small intestine

Amylose: Maltose is hydrolyzed by maltase, a brush border enzyme, forming free glucose.

Amylopectin: Maltose is hydrolyzed by maltase, a brush border enzyme, forming free glucose. The α -1,6 glycosidic bonds in limit dextrins are hydrolyzed by α -dextrinase, forming glucose.

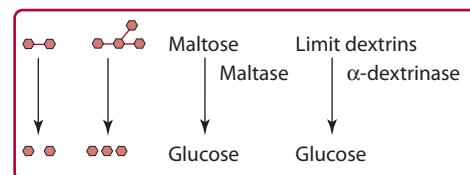
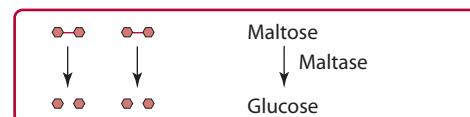


Figure 3.7 Digestion.

for example, modifying the starch by increasing the crosslinking between chains. This property of resistant starches has been the basis of commercial products as additives to processed foods.

DIGESTION OF DISACCHARIDES

Virtually no digestion of disaccharides or small oligosaccharides occurs in the mouth or stomach. In the human, digestion takes place entirely in the upper small intestine. Unlike α -amylase activity, disaccharidase activity occurs in the microvilli of the intestinal mucosal cells (the brush border), rather than in the intestinal lumen (Figures 2.10 and 2.11). Among the enzymes located on the mucosal cells are lactase, sucrase, maltase, and isomaltase. Lactase catalyzes the cleavage of lactose to equimolar amounts of galactose and glucose. As was pointed out earlier, lactose is a β 1-4 linkage, and lactase is stereospecific for this beta linkage. Lactase activity is high in infants. In most mammals, including humans, the activity of lactase decreases a few years after weaning. This diminishing activity can lead to lactose malabsorption and lactose intolerance. Lactose intolerance is particularly prevalent in African Americans, Jews, Arabs, Greeks, and some Asians. Products to reduce the effects of lactose intolerance are available on the market. These items include lactase that can be added to regular dairy products, and products that are lactose-free.

Sucrase hydrolyzes sucrose to yield one glucose and one fructose residue. Maltase hydrolyzes maltose to yield two glucose units. Isomaltase (also called α -dextrinase) hydrolyzes the α 1-6 bond of isomaltose, the branch-point disaccharide remaining from the incomplete breakdown of amylopectin; the products are two molecules of glucose (Figure 3.7).

In summary, nearly all dietary starches and disaccharides ultimately are hydrolyzed completely by specific glycosidases to their constituent monosaccharide units. Monosaccharides, together with small amounts of remaining disaccharides, can then be absorbed by the intestinal mucosal cells. The reactions involved in the digestion of starches and disaccharides are summarized in Figure 3.7.

Absorption, Transport, and Distribution

The wall of the small intestine is composed of absorptive mucosal cells and mucus-secreting goblet cells that line projections, called villi, that extend into the lumen. On the surface on the lumen side, the absorptive cells have hairlike projections called microvilli (the brush border). A square millimeter of cell surface is believed to have as many as 2×10^5 microvilli projections. The microstructure of the small intestinal wall is illustrated in Figures 2.10 and 2.11.

The anatomic advantage of the villi-microvilli structure is that it presents an enormous surface area to the intestinal contents, thereby facilitating absorption. The absorptive capacity of the human intestine has been estimated to amount to about 5,400 g/day for glucose and 4,800 g/day for fructose—a capability that would never be reached in a normal diet. Digestion and absorption of carbohydrates are so efficient that nearly all monosaccharides are usually absorbed by the end of the jejunum.

ABSORPTION OF GLUCOSE AND GALACTOSE

Glucose and galactose are absorbed into the mucosal cells by active transport, a process that requires energy and the involvement of a specific receptor. The glucose-galactose carrier has been designated sodium-glucose transporter 1 (SGLT1). It is a protein complex dependent on the Na^+/K^+ -ATPase pump, which, at the expense of ATP, furnishes energy for the transport of sugar through the mucosal cell. Glucose or galactose cannot attach to the carrier until the carrier has been preloaded with Na^+ . One glucose molecule and two sodium ions are transported into the mucosal cell at the same time. Mutation in the SGLT1 gene is associated with glucose-galactose malabsorption. An example of active transport is shown in Figure 2.18. The energy is supplied by ATP. The figure shows a carrier protein (SGLT1) loading on the lumen side of the intestinal mucosa cell and releasing it inside the cell.

Glucose appears to leave the mucosal cell at the basolateral surface by three routes. Approximately 15% leaks back across the brush border into the intestinal lumen, about 25% diffuses through the basolateral membrane into the circulation, and the major portion (~60%) is transported from the cell into the circulation by a carrier, GLUT2 (discussed in a later section in this chapter), in the serosal membrane. A small portion of the available glucose may be used by the mucosal cell for its energy needs.

ABSORPTION OF FRUCTOSE

Fructose is transported into the mucosal cell by a specific facilitative transporter GLUT5. The entry of fructose into the cell is independent of glucose concentration and occurs even in the presence of high concentrations of glucose [2]. This transport is independent of the active, Na^+ -dependent transport of glucose, but the rate of uptake is much slower than that of both glucose and galactose. A large proportion of human subjects studied showed an inability to absorb completely doses of fructose in the range of 20 to 50 g [3]. Fructose is transported from the mucosal cell by GLUT2, the same transporter that moves glucose out of the cell. The facilitative transport process can proceed only down a concentration gradient. Fructose is very efficiently absorbed by the liver, where it is phosphorylated

and trapped. This immediate reaction of fructose with phosphate as it enters the liver cell results in virtually no circulating fructose in the bloodstream, which ensures a downhill concentration gradient (a higher concentration in the intestinal mucosa and no circulating fructose).

Although fructose absorption takes place more slowly than that of glucose or galactose, which are actively absorbed, it is absorbed faster than sugar alcohols such as sorbitol and xylitol, which are absorbed purely by passive diffusion. The extent of the contribution of active transport and facilitative diffusion to fructose absorption has not been established, though both systems are saturable. This circumstance accounts for the observations that fructose absorption is limited in nearly 60% of normal adults and that intestinal distress, symptomatic of malabsorption, frequently appears following ingestion of 50 g of pure fructose [4]. This level of intake is commonly found in high-fructose syrups used as sweeteners. Interestingly, coconsumption of glucose with fructose accelerates the absorption of fructose and raises the threshold level of fructose ingestion at which malabsorption symptoms appear [4]. This observation suggests that the pair of monosaccharides might be absorbed by a so-called disaccharidase-related transport system designed to transport the hydrolytic products of sucrose [3].

MONOSACCHARIDE TRANSPORT AND CELLULAR UPTAKE

Following transport across the wall of the intestine, the monosaccharides enter the portal circulation, where they are carried directly to the liver. The liver is the major site of metabolism of galactose and fructose, which are readily taken up by the liver through specific hepatocyte receptors. They enter these liver cells by facilitated transport and subsequently are metabolized. Both fructose and galactose can be converted to glucose derivatives through pathways that are described later in this chapter. Once fructose and galactose are converted to glucose derivatives they have the same fate as glucose and can be stored as liver glycogen, or catabolized for energy according to the liver's energy demand. The blood levels of galactose and fructose are not directly subject to the strict hormonal regulation that is such an important part of glucose homeostasis. However, if their dietary intake is a significantly higher than the normal percentage of total carbohydrate intake, they may be regulated indirectly as glucose because of their metabolic conversion to that sugar.

Glucose is nutritionally the most important monosaccharide because it is the exclusive constituent of the starches and also occurs in each of three major disaccharides (Figure 3.1). Like fructose and galactose, glucose is extensively metabolized in the liver, but its removal by that organ is not as complete as in the case of fructose

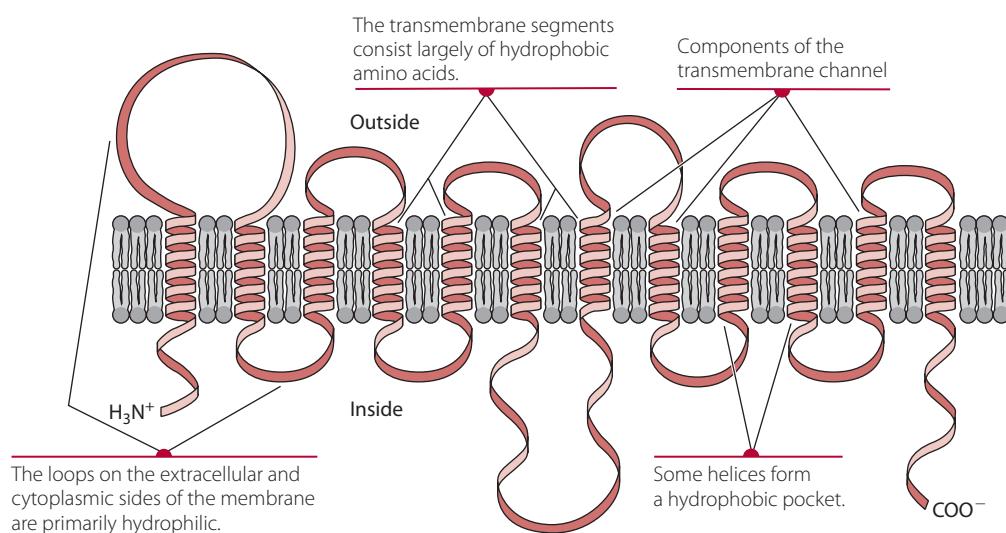
and galactose. The remainder of the glucose passes into the systemic blood supply and is then distributed among other tissues, such as muscle, kidney, and adipose tissue. Glucose enters the cells in these organs by facilitated transport. In skeletal muscle and adipose tissue the process is insulin dependent, whereas in the liver it is insulin independent. Because of the nutritional importance of glucose, the facilitated transport process by which it enters the cells of certain organs and tissues warrants a closer look. The following section explores the process in greater detail.

GLUCOSE TRANSPORTERS

Glucose is effectively used by a wide variety of cell types under normal conditions, and its concentration in the blood must be precisely controlled. Glucose plays a central role in metabolism and cellular homeostasis. Most cells in the body are dependent upon a continuous supply of glucose to supply energy in the form of ATP. The symptoms associated with diabetes mellitus are a graphic example of the consequences of a disturbance in glucose homeostasis. The cellular uptake of glucose requires that it cross the plasma membrane of the cell. The highly polar glucose molecule cannot move across the cellular membrane by simple diffusion because the highly polar molecule cannot pass through the nonpolar matrix of the lipid bilayer. For glucose to be used by cells, an efficient transport system for moving the molecule into and out of cells is essential. In certain absorptive cells, such as epithelial cells of the small intestine and renal tubule, glucose crosses the plasma membrane (actively) against a concentration gradient, pumped by an Na^+/K^+ -ATPase symport system (SGLT) described above and in Chapter 1. However, glucose is passively admitted to nearly all cells in the body by a carrier-mediated transport mechanism that does not require energy. The family of protein carriers involved in this process are called glucose transporters, abbreviated GLUT.

GLUT Isoforms

A total of twelve individual transport proteins have been identified along with the genes that code for them. The genome project has aided in this identification because considered collectively, all transporter proteins share a common structure, and similar sequences in the genes code for them. About 28% of the amino sequences are common within the family of transport proteins. Each GLUT is an integral protein, penetrating and spanning the lipid bilayer of the plasma membrane. Most transporters, in fact, span the membrane several times. They are oriented so that hydrophilic regions of the protein chain protrude into the extracellular and cytoplasmic media, while the hydrophobic regions traverse the membrane, juxtaposed with the membrane's lipid matrix. A model for a glucose transporter,

**Figure 3.8**

A model for the structural orientation of the glucose transporter in the erythrocyte membrane.

reflecting this spatial arrangement of the molecule, is illustrated in Figure 3.8. In its simplest form, a transporter:

- has a specific combining site for the molecule being transported
- undergoes a conformational change upon binding the molecule, allowing the molecule to be translocated to the other side of the membrane and released
- has the ability to reverse the conformational changes without the molecule's being bound to the transporter, so that the process can be repeated

Twelve isoforms of glucose transporters have been described. They are listed in Table 3.2, which includes the primary sites of expression. All cells express at least one GLUT isoform on their plasma membranes. The different

isoforms have distinct tissue distribution and biochemical properties, and they contribute to the precise disposal of glucose according to varying physiological conditions [5,6].

Specificity of GLUTs

The GLUT1 is responsible for the basic supply of glucose to the cells. GLUT1 is expressed in erythrocytes and endothelial cells of the brain. GLUT2 is a low affinity transporter with predominate expression in the β -cells of the pancreas, liver, and kidney. As discussed previously, this transporter is involved in the transport of glucose from the intestinal mucosal cell into the portal blood. GLUT2 is also able to transport fructose out of the intestinal mucosal cell. The rate of transport is highly dependent on the blood glucose concentration.

GLUT3 is a high-affinity glucose transporter with predominate expression in those tissues that are highly dependent upon glucose such as the brain. GLUT4, in contrast, is quite sensitive to insulin, and its concentration on the plasma membrane increases dramatically in response to the hormone. The increase in the membrane transporter population is accompanied by an accelerated increase in the uptake of glucose by the insulin-stimulated cells. The presence of GLUT4 in skeletal muscle and in adipose tissue is what makes these tissues responsive to insulin. Liver, brain, and erythrocytes lack the GLUT4 isoform and therefore are not sensitive to insulin. A feature of type 2 diabetes, described in the Chapter 7 Perspective "Diabetes: Metabolism Out of Control," is a resistance to insulin. The molecular basis for type 2 diabetes, insulin resistance, and the metabolic syndrome are discussed in Chapter 7. GLUT5 is specific for the transport of fructose. The physiological actions of some of the newly discovered GLUTs have been studied using molecular biology techniques. Because the genes for these proteins are known, a technique that blocks the gene's expression in experimental animals such as the mouse is used to determine what effect the absence of the GLUT has on the animal. GLUTs have also been shown to

Table 3.2 Glucose Transporters (GLUT)

Transporter Protein	Insulin Regulatable	Major Sites of Expression
GLUT1	No	Erythrocytes, blood brain barrier, placenta, fetal tissues in general
GLUT2	No	Liver, β -cells of pancreas, kidney, small intestine
GLUT3	No	Brains (neurons)
GLUT4*	Yes	Muscle, heart, brown and white adipocytes
GLUT5	No	Intestine, testis, kidney
GLUT6	No	Spleen, leukocytes, brain
GLUT7	No	Unknown
GLUT8	No	Testis, blastocyst, brain
GLUT9	No	Liver, kidney
GLUT10	No	Liver, pancreas
GLUT11	No	Heart, muscle
GLUT12	No	Heart, prostate

*Note that GLUT4 is regulatable by insulin.

Source: Joost HG, Thorens B. The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). Modified from Molecular Membrane Biology, 2001, 18, 247–256.

be detectors of the glucose levels in certain tissue such as the islet cells of the pancreas. An excellent review of the GLUT isoform family is found in [5].

Synthesis and storage of the insulin-responsive transporter GLUT4, as for the other transporter isoforms, occur as described in Chapter 1 for all proteins. Following its synthesis from mRNA on the ribosomes of the rough endoplasmic reticulum, the transporter enters the compartments of the Golgi apparatus, where it is ultimately packaged in tubulovesicular structures in the *trans*-Golgi network. In the basal, unstimulated state of the adipocyte, GLUT4 resides in these structures as well as, to some extent, in small cytoplasmic vesicles [7]. This subcellular distribution of GLUT4 is also found in skeletal muscle cells [8]. Blood glucose levels are maintained within a narrow range by a balance between glucose absorption from the intestine, production by the liver and uptake and metabolism by the peripheral tissue. Insulin is a hormone that plays a central role in the level of blood glucose during periods of feeding and fasting.

INSULIN

This section covers the role of insulin in the cellular absorption of glucose. Insulin is a very powerful anabolic hormone and is involved in glucose, lipid, and amino acid/protein synthesis and storage. Insulin's roles in amino acid and lipid metabolism are discussed further in Chapters 6 and 7. In general, insulin increases the expression or activity

of enzymes that catalyze the synthesis of glycogen, lipids, and proteins. It also inhibits the expression or activity of enzymes that catalyze the catabolism of glycogen, lipids, and amino acids. Figure 3.9 illustrates the anabolic and catabolic effects of insulin on glucose and glycogen, fatty acids and triacylglycerols, and amino acids and proteins.

Much has been learned about mechanism of insulin's action in the last few years in controlling blood glucose levels. We have a much better understanding of insulin resistance, type 2 diabetes, and its complications. Insulin plays a vital role in regulating blood glucose levels.

Role in Cellular Glucose Absorption

When blood glucose levels are elevated, insulin is released by the β -cells of the pancreas. Insulin stimulates the uptake of glucose by muscle and adipose and also inhibits the synthesis of glucose (gluconeogenesis) by the liver. Insulin binds to a specific receptor located on the cell membrane of muscle and adipose tissue and stimulates the tubulovesicle-enclosed GLUT4 transports to be translocated to the plasma membrane. Insulin functions through a second messenger system and belongs to a subfamily of receptor tyrosine kinases that include insulin receptor-related receptor (IRR) and insulin-like growth factor (IGF) [9] (see Figure 3.10). The figure does not show all of the detail but does indicate that insulin and tyrosine kinase reactions effect general gene expression, cell growth and differentiation, glucose metabolism, glycogen/lipid/protein

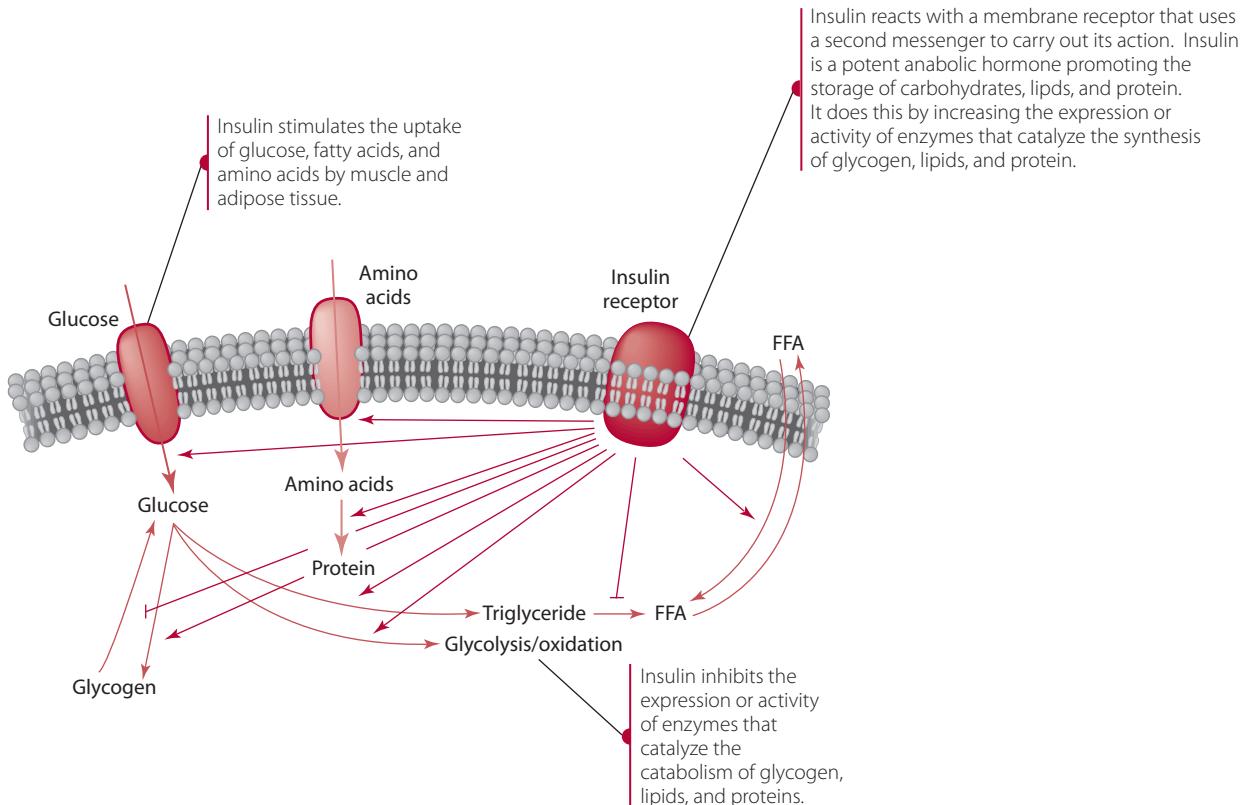


Figure 3.9 Insulin regulation of metabolism.

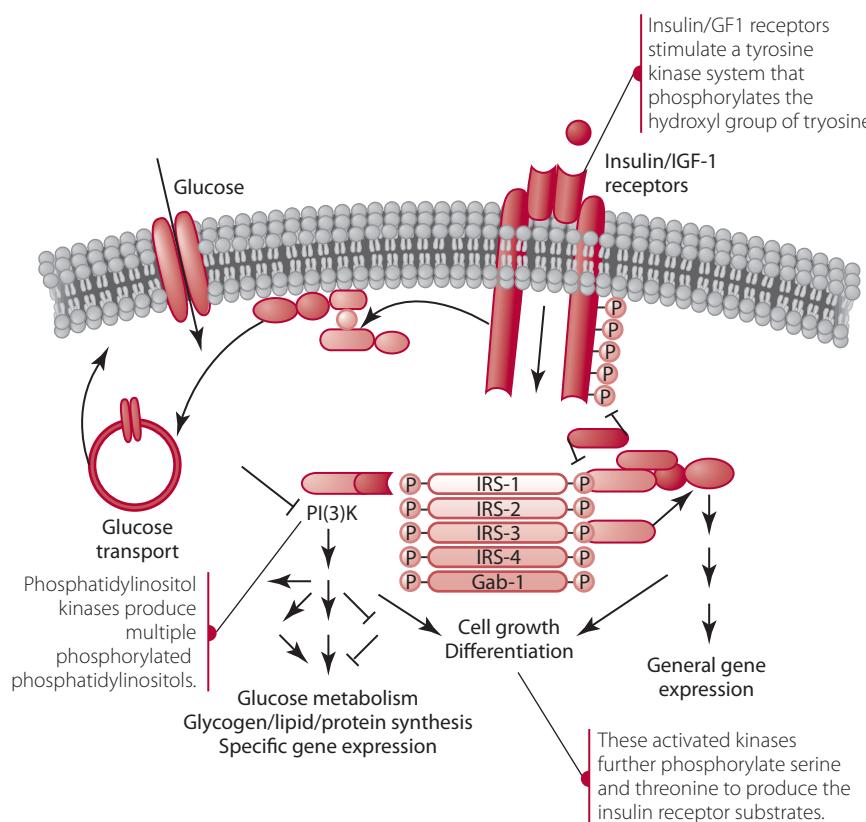


Figure 3.10 Mechanism of insulin action.

synthesis, specific gene expression, as well as glucose transport. The insulin/IGF-1 receptor kinase acts on at least nine different substrates. The kinase phosphorylates the hydroxyl group of tyrosine in a variety of proteins that include G-proteins and certain enzymes, such as phosphotyrosine phosphatase and cytoplasmic tyrosine kinase. The phosphorylation/dephosphorylation reactions regulate the activity of these enzymes and, in some cases, their subcellular location. Following the phosphorylation of the insulin receptor, a series of serine-threonine phosphorylations take place to produce insulin-receptor substrates, labeled as IRS1 through IRS4 in Figure 3.10. These reactions are coupled with several additional protein kinase systems. One of these protein kinases involves phosphatidylinositol 3-kinase (PI 3-kinase), which converts phosphatidylinositol to 3,4 bisphosphate to phosphatidylinositol 3,4,5 triphosphate ($\text{Pi}_3,4,5\text{P}_3$). The details of the individual phosphorylations go beyond the scope of this text and are not shown in Figure 3.10. These reactions are not necessary for the understanding of how insulin carries out its functions. Later chapters provide the ways in which the molecular mechanism of insulin resistance involves phosphorylation reactions. Though much has been learned, insulin's complete molecular mechanism of action and the development of insulin resistance are not fully understood [10].

The endothelial tissue of which blood vessel walls are constructed is freely permeable to metabolites such as glucose. Some tissues, most notably the brain, possess an

additional layer of epithelial tissue between the blood vessel and the cells of the brain. Unlike endothelium, epithelial layers are not readily permeable, and the passage of metabolites through them requires active transport or facilitated diffusion. For this reason, epithelium is called the blood tissue barrier of the body. Among the blood tissue barriers studied—including those of brain, cerebrospinal fluid, retina, testes, and placenta—GLUT1 appears to be the prime isoform for glucose transport [11].

In summary, glucose is transported into cells in the body by the isoforms of glucose transport proteins, and cells of the various organs have specific glucose transporters associated with them. The level of GLUT4, found primarily in skeletal muscle and adipose tissue, is insulin dependent. Insulin is an important hormone in signaling whether the body is in times of plenty or not. In times of plenty, insulin stimulates glycogen synthesis in the liver and fatty acid synthesis in adipose tissue.

MAINTENANCE OF BLOOD GLUCOSE LEVELS

Maintenance of normal blood glucose concentration is an important homeostatic function and is a major function of the liver. Regulation is the net effect of the organ's metabolic processes that remove glucose from the blood, either for glycogen synthesis or for energy release, and of processes that return glucose to the blood, such as glycogenolysis and gluconeogenesis. These pathways, which are examined in

Table 3.3 Metabolic Pathways of Carbohydrate Metabolism

Glycogenesis	Making of glycogen
Glycogenolysis	Breakdown of glycogen
Glycolysis	Oxidation of glucose
Glyconeogenesis	Production of glucose from noncarbohydrate intermediates
Hexose monophosphate shunt	Production of 5-carbon monosaccharides and NADPH
Tricarboxylic acid cycle (TCA)	Oxidation of pyruvate and acetyl CoA

detail in the section “Integrated Metabolism in Tissues,” are hormonally influenced, primarily by the antagonistic pancreatic hormones insulin and glucagon and the glucocorticoid hormones of the adrenal cortex. The rise in blood glucose following the ingestion of carbohydrate, for example, triggers the release of insulin while reducing the secretion of glucagon. These changes in the two hormone levels increase the uptake of glucose by muscle and adipose tissue, returning blood glucose to homeostatic levels. A fall in blood glucose concentration, conversely, signals a reversal of the pancreatic hormonal secretions—that is, decreased insulin and increased glucagon release. In addition, an increase in the secretion of glucocorticoid hormones, primarily cortisol, occurs in answer to—and to offset—a falling blood glucose level. Glucocorticoids cause increased activity of hepatic gluconeogenesis, a process described in detail in a later section. Several terms used in carbohydrate metabolism sound and appear to be very similar but are in fact quite different. Table 3.3 provides a list of these terms and their definition to provide a path for better understanding glucose metabolism.

Glycemic Response to Carbohydrates

The rate at which glucose is absorbed from the intestinal tract appears to be an important parameter in controlling the homeostasis of blood glucose, insulin release, obesity, and possibly weight loss. The intense research of the last few years appears to give the concept of glycemic index and glycemic load scientific validity [12]. Current research suggests a role for elevated blood glucose in the development of chronic diseases and obesity. The concept of glycemic index and glycemic load is discussed in this section. Their role in insulin resistance and type 2 diabetes is covered in Chapters 7 and 8. See also the Perspective on diabetes following Chapter 7.

GLYCEMIC INDEX

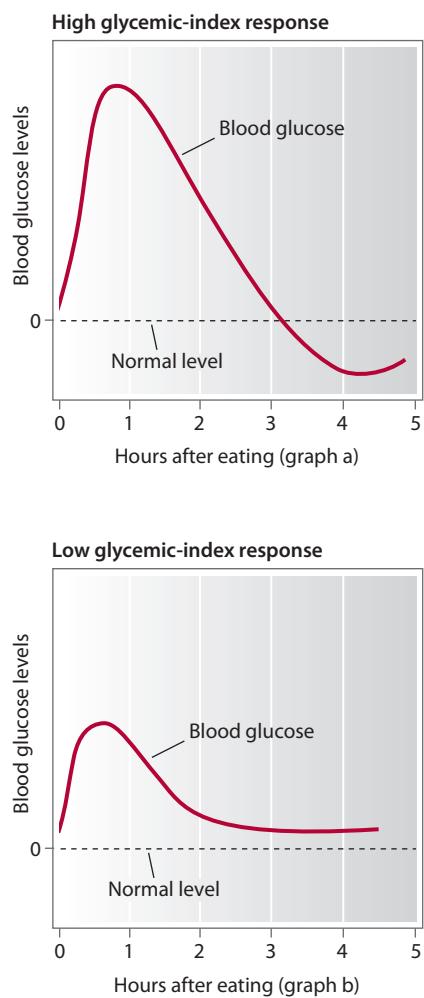
An alternative way to classify dietary carbohydrates is by their ease of absorption and their effect on the elevation of blood glucose levels. The implications of consuming high-glycemic index foods for chronic disease and obesity have recently been reviewed [13–15]. These reviews suggest

that the glycemic index and glycemic load (defined below) offer a way to examine the relative risks of diets designed to prevent coronary heart disease (CHD) and obesity.

The effect that carbohydrate-containing foods have on blood glucose concentration, called the glycemic response of the food, varies with the time it takes to digest and absorb the carbohydrates in that food. Some foods cause a rapid rise and fall in blood glucose levels, whereas others cause a slower and more extended rise with a lower peak level and a gradual fall. The concept of the glycemic index of a food was developed to provide a numerical value to represent the effect of the food on blood glucose levels. It provides a quantitative comparison between foods. Glycemic index is defined as the increase in blood glucose level over the baseline level during a 2-hour period following the consumption of a defined amount of carbohydrate (usually 50 g) compared with the same amount of carbohydrate in a reference food. Earlier studies typically used glucose as the test food. More recently, white bread is being used, and white bread is assigned a score of 100. In practice, the glycemic index is measured by determining the elevation of blood glucose for 2 hours following ingestion. The area under the curve after plotting the blood glucose level following ingestion of the reference food is divided by the area under the curve for the reference food times 100 (Figure 3.11). If glucose is used as the reference food, it is arbitrarily assigned a glycemic index of 100. With glucose as the reference food white bread has a glycemic index of about 71. The use of white bread as the reference assigns the glycemic index of white bread of 100. The use of white bread as the standard causes some foods to have a glycemic index of greater than 100. One criticism of glycemic index is the variation of glycemic index for apparently similar foods. One cause could be the difference in the reference food used. This variation may reflect methodological differences as well as differences in the food preparation and the ingredients used in preparing the food. The difference could also reflect real differences in the biological variety of the food. For instance, the glycemic index for a baked russet potato is 76.5 and for an instant mashed potato is 87.7 (using glucose as the reference food) [16]. Even the temperature of the food can make a difference: A boiled red potato hot has a glycemic index of 89.4, and the same potato cold has a glycemic index of 56.2 (Table 3.4).

GLYCEMIC LOAD

The question has been raised as to whether the glycemic index has any practical relevance, because we do not eat a single food but meals that are made up of a number of foods. To address this question, the concept of glycemic load was introduced. Glycemic load considers both the quantity and the quality of the carbohydrate in a meal. The glycemic load (GL) equals the glycemic index times the grams of carbohydrate in a serving of the food. The higher the GL, the greater the expected elevation in blood glucose and in



Calculation of Glycemic Index

- ① The elevation in blood glucose level above the baseline following consumption of a high glycemic-index food or 50 g of glucose in a reference food (glucose or white bread). The glycemic index of the reference food is by definition equal to 100 (graph a).
- ② The elevation of blood glucose levels above the baseline following the intake of 50 g of glucose in a low glycemic-index food (graph b).
- ③ The glycemic index is calculated by dividing the area under the curve for the test food by the area under the curve by the reference times 100.

Figure 3.11 Blood glucose changes following carbohydrate intake (glycemic index).

the insulinogenic effect of the food. Long-term consumption of a diet with a relatively high GL is associated with an increased risk of type 2 diabetes and coronary heart disease [17]. The literature suggests that the longer and higher the elevation of blood glucose, the greater the risk of developing chronic diseases and obesity [13,14].

Table 3.4 Glycemic Index of Common Foods with White Bread and Glucose Used as the Reference Food

Food Tested	Glycemic Index	
	White Bread = 100	Glucose = 100
White Bread ¹	100	71
Baked Russet Potato ¹	107.7	76.5
Instant Mashed Potatoes ¹	123.5	87.7
Boiled Red Potato (hot) ¹	125.9	89.4
Boiled Red Potato (cold) ¹	79.2	56.2
Bran Muffin ²	85	60
Coca Cola ²	90	63
Apple Juice, Unsweetened ²	57	40
Tomato Juice ²	54	38
Bagel ²	103	72
Whole-Meal Rye Bread ²	89	62
Rye-Kernal Bread ² (Pumpernickel)	58	41
Whole Wheat Bread ²	74	52
All-Bran Cereal ²	54	38
Cheerios ²	106	74
Corn Flakes ²	116	81
Raisin Bran ²	87	61
Sweet Corn ²	86	60
Couscous ²	81	61
Rice ²	73	51
Brown Rice ²	72	50
Ice Cream ²	89	62
Soy Milk ²	63	44
Raw Apple ²	57	40
Banana ²	73	51
Orange ²	69	48
Raw Pineapple ²	94	66
Baked Beans ²	57	40
Dried Beans ²	52	36
Kidney Beans ²	33	23
Lentils ²	40	28
Spaghetti, Durum Wheat (boiled) ²	91	64
Spaghetti, Whole Meal (boiled) ²	32	46
Sucrose ²	83	58

¹ Fernandes G, Velangi A, Wolever T. Glycemic Index of Potatoes Commonly Consumed in North America. *J Am Diet Assoc* 2005; 105:557-62.

² From Foster-Powell, K., Holt, S., Brand-Miller, J., "International Table of Glycemic Index and Glycemic Load Values." *American Journal of Clinical Nutrition*, 2002; 76:5-56. Reprinted by permission.

Many published tables provide the glycemic index for different foods. The most complete is an international table [18]. Selected examples from this publication have been reproduced in Table 3.4 along with the glycemic index of potatoes. Remember that the food products differ in different regions of the world. The glycemic indices listed in Table 3.4 are intended to be used to show trends and not to prepare diets. Glycemic index and glycemic load have proven useful in evaluating the risk of developing chronic disease and obesity. One of the risk factors for these chronic diseases appears to be related to the degree of blood glucose elevation and the length of time glucose levels are elevated.

Integrated Metabolism in Tissues

The metabolic fate of the monosaccharides depends to a great extent on the body's energy needs at the time. The activity of certain metabolic pathways is regulated according to these needs in such a way that some may be stimulated, and others may be suppressed. The major regulatory mechanisms are hormonal (involving the action of hormones such as insulin, glucagon, epinephrine, and the corticosteroid hormones) and allosteric enzyme activation or suppression (see Chapter 1). Allosteric enzymes provide regulation for some pathways because their activities can be altered by compounds called modulators. A negative modulator of an allosteric enzyme reduces the activity of the enzyme and slows the velocity of the reaction it catalyzes, whereas a positive modulator increases the activity of an allosteric enzyme and thus increases the velocity of the reaction. The effect of a modulator, whether negative or positive, is exerted on its allosteric enzyme as a result of changes in the concentration of the modulator. The mechanisms by which metabolism is regulated, including induction, post-translational modification, and translocation, are discussed in detail in the section "Regulation of Metabolism."

The metabolic pathways of carbohydrate use and storage (Table 3.3) consist of **glycogenesis** (the making of glycogen), glycogenolysis (the breakdown of glycogen), and **glycolysis** (the oxidation of glucose), the **hexosemonophosphate shunt** (the production of 5-carbon monosaccharides and NADPH), the **tricarboxylic acid cycle** (TCA cycle) (oxidation of pyruvate and acetyl CoA) sometimes called the Krebs cycle or the citric acid cycle, and **gluconeogenesis** (the making of glucose from noncarbohydrate precursors). An integrated overview of these pathways is given in Figure 3.12. A detailed review of the pathways' intermediary metabolites, sites of regulation, and, most important, functions in the overall scheme of things are considered in the sections

that follow. The detailed pathways showing the names of the chemicals and providing their structures are shown in the later figures. These are followed with a discussion of the individual reactions and additional comments that are particularly significant from a nutritional standpoint are provided following the reactions in the pathway. Because of the central role of glucose in carbohydrate nutrition, its metabolic fate is featured here. The entry of fructose and galactose into the metabolic pathways is introduced later in the discussion.

GLYCOGENESIS

The term **glycogenesis** refers to the pathway by which glucose ultimately is converted into glycogen. This pathway is particularly important in hepatocytes because the liver is a major site of glycogen synthesis and storage. Glycogen accounts for as much as 7% of the wet weight of the liver. Liver glycogen can be broken down to glucose and reenter the bloodstream. Therefore, it plays an important role in maintaining blood glucose homeostasis. The other major sites of glycogen storage are skeletal muscle and, to a much lesser extent, adipose tissue. In human skeletal muscle, glycogen generally accounts for a little less than 1% of the wet weight of the tissue. Most of the body's glycogen (about 75%) is stored in the muscle, because the muscle makes up a much greater portion of the body's weight than the liver does. Liver glycogen is more important in maintaining blood glucose homeostasis. The glycogen stores in muscle can be used as an energy source in that muscle fiber when the body is confronted by an energy demand such as physical exertion. The glycogenic pathway (the synthesis of glycogen) therefore is vitally important in ensuring a reserve of instant energy. The initial part of the glycogenic pathway is illustrated in Figure 3.13.

Glucose is first phosphorylated upon entering the cell, producing a phosphate ester at the 6-carbon of the glucose. In muscle cells, the enzyme catalyzing this phosphate transfer from ATP is hexokinase. Hexokinase is an allosteric enzyme that is negatively modulated by the product of the reaction, glucose 6-phosphate. That means when the muscle cell has adequate glucose 6-P additional glucose is phosphorylated more slowly. Glucose phosphorylation in the liver is catalyzed primarily by glucokinase (sometimes called hexokinase D). Although the reaction product, glucose 6-phosphate, is the same, interesting differences distinguish it from hexokinase. For example, hexokinase is negatively modulated by glucose 6-phosphate, whereas glucokinase is not. This characteristic allows excess glucose entering the liver cell to be phosphorylated quickly and encourages glucose entry when blood glucose levels are elevated. Also, glucokinase has a much higher K_m than hexokinase, meaning that it can convert glucose to its phosphate form at a higher velocity

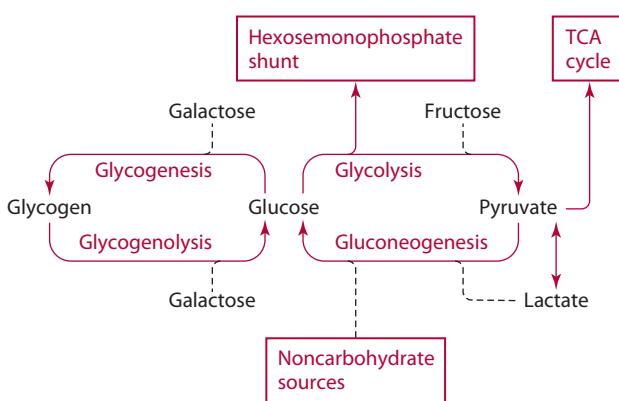


Figure 3.12 Reaction of glycogenesis. The formation of glycogen from glucose.

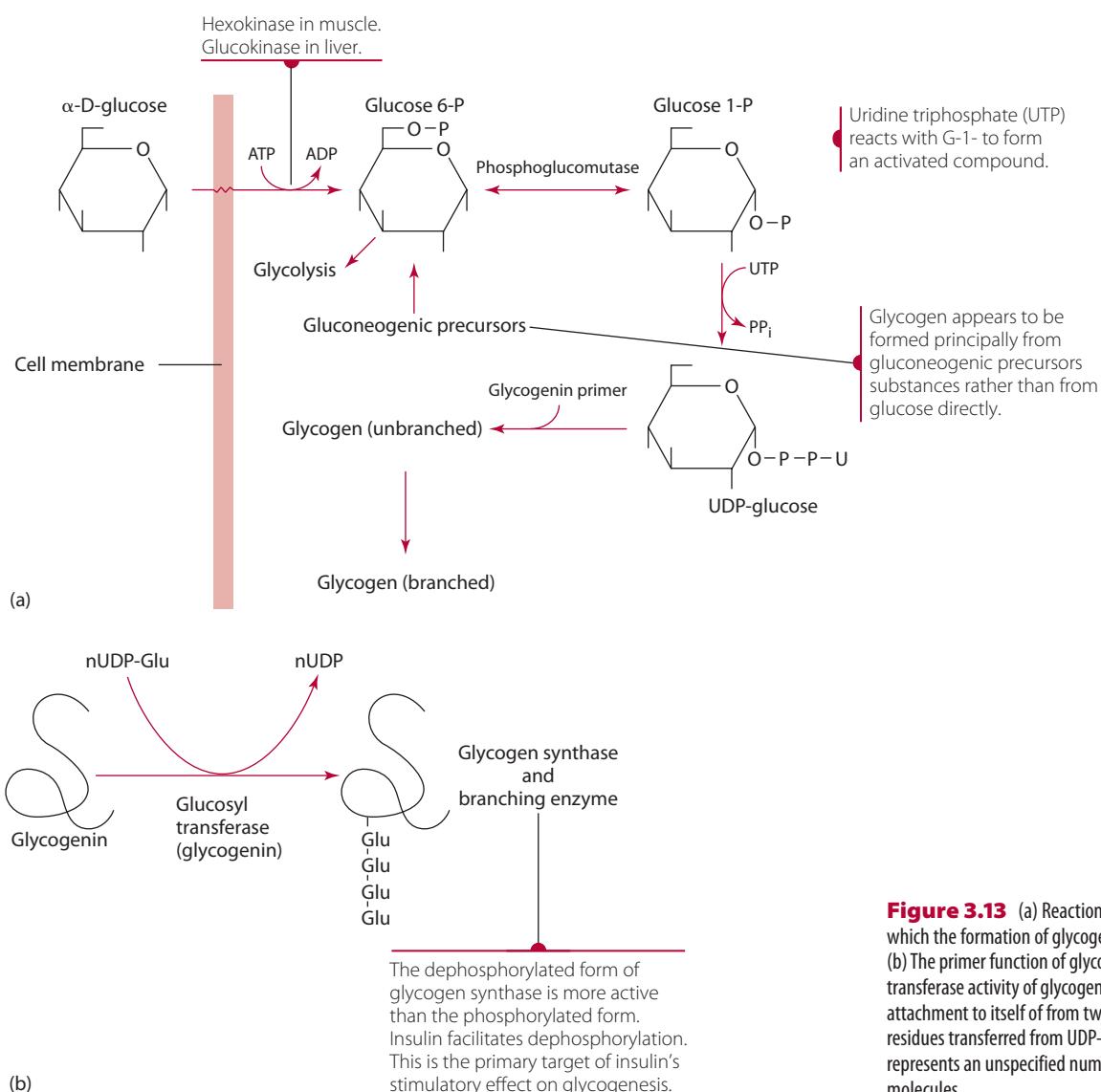


Figure 3.13 (a) Reactions of glycogenesis, by which the formation of glycogen from glucose occurs. (b) The primer function of glycogenin. The glucosyl transferase activity of glycogenin catalyzes the attachment to itself of from two to seven glucose residues transferred from UDP-glucose. The letter *n* represents an unspecified number of UDP-glucose molecules.

should the cellular concentration of glucose rise significantly (e.g., after a carbohydrate-rich meal). In muscle, the much lower K_m of hexokinase indicates that it is catalyzing at maximum velocity, even at average glucose concentrations. Glycogen synthesis (glycogenesis) is initiated by the presence of glucose 6-phosphate. The phosphorylation of glucose as it enters the liver cell keeps the level of free glucose low, which enhances the entry of glucose into the liver cell due to the concentration gradient between blood and inside of liver cell. Therefore, the liver has the capacity to reduce blood glucose concentration when it becomes high. Remember the liver is not dependent upon insulin for glucose transport into the cell, but glucokinase is inducible by insulin. Insulin blood levels are increased by elevated blood glucose levels. In diabetes mellitus, type 1 (see the Chapter 7 Perspective on diabetes), glucokinase activity is below normal values because type 1 diabetes

patients have low insulin levels, and the glucokinase is not induced. The low glucokinase activity contributes to the liver cell's inability to rapidly take up and metabolize glucose, which occurs even though GLUT2 of the liver is not regulated by insulin. The concept of K_m and its significance as it applies to this reaction were discussed in Chapter 1. The hexokinase/glucokinase reaction is energy consuming because the glucose is activated (phosphorylated) at the expense of ATP.

The phosphate is transferred from the 6-carbon of the glucose to the 1-carbon in a reaction catalyzed by the enzyme phosphoglucomutase. Nucleoside triphosphates other than ATP sometimes function as activating substances in intermediary metabolism. In the next reaction, energy derived from the hydrolysis of the α - β -phosphate anhydride bond of uridine triphosphate (UTP to UMP) allows the resulting uridine monophosphate to be coupled to

the glucose 1-phosphate to form uridine diphosphate glucose (UDP-glucose). Glucose is incorporated into glycogen as UDP-glucose. The reaction is catalyzed by glycogen synthase and requires some preformed glycogen as a primer, to which the incoming glucose units can be attached. The initial glycogen is formed by binding a glucose residue to a tyrosine residue of a protein called glycogenin. In this case, glycogenin acts as the primer. Additional glucose residues are attached by glycogen synthase to form chains of up to eight units. The role of glycogenin in glycogenesis has been reviewed [19]. In muscle the protein stays in the core of the glycogen molecule, but in the liver more glycogen molecules than glycogenin molecules are present, so the glycogen must break off of the protein. Glycogen synthase exists in an active (dephosphorylated) form and a less active (phosphorylated) form. Insulin facilitates glycogen synthesis by stimulating the dephosphorylation of glycogen synthase. The glycogen synthase reaction is the primary target of insulin's stimulatory effect on glycogenesis.

When six or seven glucose molecules are added to the glycogen chain the branching enzyme transfers them to a C(6)—OH group (Figure 3.14). Glycogen synthase cannot form the α 1-6 bonds of the branch points. This action is left to the branching enzyme, which transfers a seven residue oligosaccharide segment from the end of the main glycogen chain to carbon number 6 hydroxyl groups throughout the chain (Figure 3.14). Branching within the glycogen molecule is very important, because it increases the molecule's solubility and compactness. Branching also makes available many nonreducing ends of chains from which glucose residues can be cleaved rapidly and used for energy, in the process known as glycogenolysis and described in the following section. The overall pathway of glycogenesis, like most synthetic pathways, consumes energy, because an ATP and a UTP are consumed for each molecule of glucose introduced.

GLYCOGENOLYSIS

The potential energy of glycogen is contained within the glucose residues that make up its structure. In accordance with the body's energy demands, the residues can be systematically cleaved one at a time from the ends of the glycogen branches and routed through energy-releasing pathways. The breakdown of glycogen into individual glucose units, in the form of glucose 1-phosphate, is called glycogenolysis. Like its counterpart, glycogenesis, glycogenolysis is regulated by hormones, most importantly glucagon (of pancreatic origin) and the catecholamine hormone epinephrine (produced in the adrenal medulla). Both of these hormones stimulate glycogenolysis and are directed at the initial reaction, glycogen phosphorylase. Both hormones function through the second messenger cAMP, which regulates phosphorylation state of the enzymes involved. Glucagon and epinephrine function

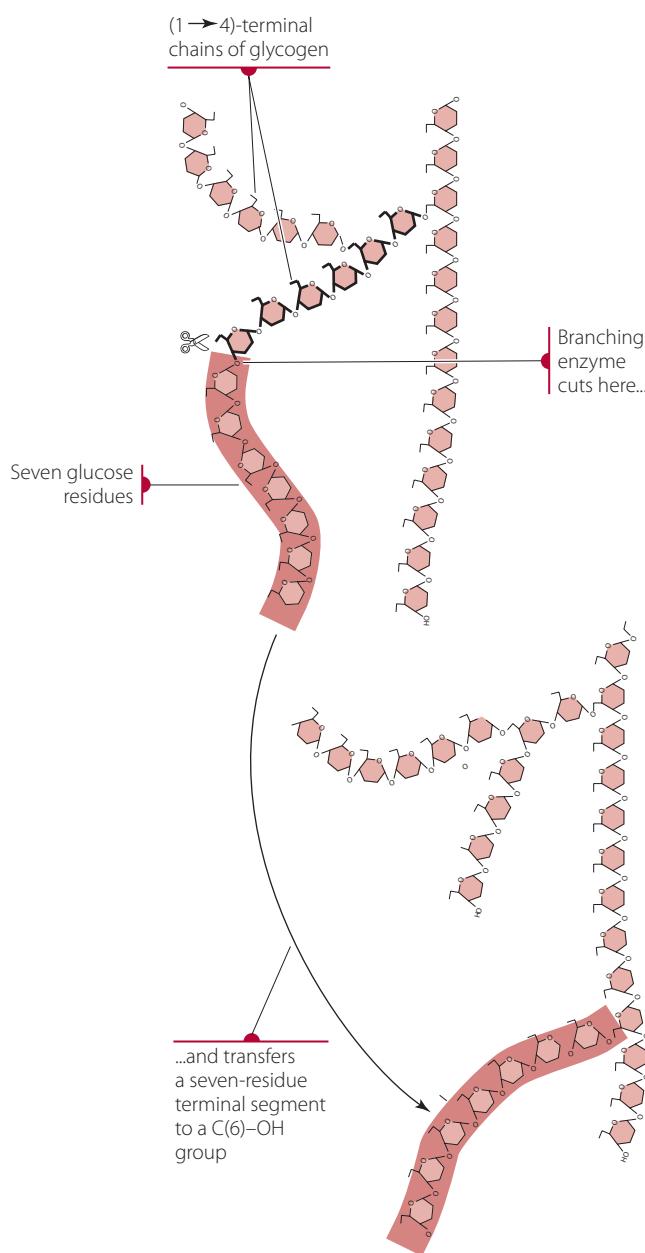


Figure 3.14 Formation of glycogen branches by the branching enzyme.

antagonistically to insulin in regulating the balance between free (glucose) and stored glucose (glycogen). The steps involved in glycogenolysis are shown in Figure 3.15.

When energy is needed, individual glucose units are released from glycogen by a **phosphorolysis** process by which the glycosidic bonds are cleaved by phosphate addition. The products of one such cleavage reaction are glucose 1-phosphate and the remainder of the intact glycogen chain minus the one glucose residue. The reaction is catalyzed by glycogen phosphorylase, an important site of metabolic regulation by both hormonal and allosteric enzyme modulation. Glycogen phosphorylase can exist as either phosphorylase a (a phosphorylated active form) or phosphorylase b (a dephosphorylated, inactive form).

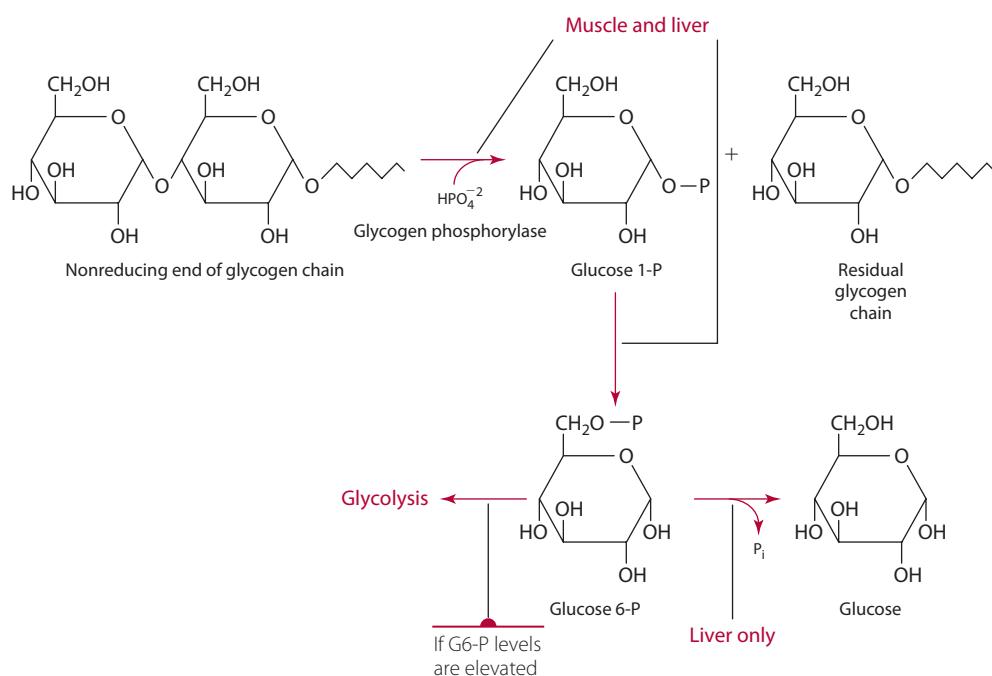


Figure 3.15 The reactions of glycogenolysis, by which glucose residues are sequentially removed from the nonreducing ends of glycogen segments.

The two forms are interconverted by other enzymes, which can either attach phosphate groups to the phosphorylase enzyme or remove phosphate groups from it. The enzyme catalyzing the phosphorylation of phosphorylase b to its active “a” form is called phosphorylase b kinase. The enzyme that removes phosphate groups from the active “a” form of phosphorylase, producing the inactive “b” form, is called phosphorylase a phosphatase. The rate of glycogen breakdown to glucose 1-phosphate therefore depends on the relative activity of phosphorylase a and phosphorylase b.

The regulation of phosphorylase activity in the breakdown of liver and muscle glycogen is complex. It can involve covalent regulation, which is the phosphorylation-dephosphorylation regulation just described, and may also involve allosteric regulation by modulators. These and other mechanisms of regulation are broadly reviewed in the section “Regulation of Metabolism.”

- *Covalent regulation* is strongly influenced by the hormones glucagon and epinephrine. Glucagon acts in the liver and adipose tissue and epinephrine acts in the liver and muscle. These hormones exert their effect by stimulating phosphorylase b kinase, thereby promoting formation of the more active (“a”) form of the enzyme. This hormonal activation of phosphorylase b kinase is mediated through cAMP, the cellular concentration of which is increased by the action of the same hormones, epinephrine and glucagon.

- *Allosteric regulation* of phosphorylase generally involves the positive modulator AMP, which induces

a conformational change in the inactive “b” form, resulting in a fully active “b” form. ATP competes with AMP for the allosteric site of the enzyme. High ATP levels prevent the shift to the enzyme’s active form and tend to keep it in its inactive form. No covalent (phosphorylation) regulation is involved in allosteric modulations.

The interconversion of phosphorylase a and phosphorylase b, along with the active and inactive forms of phosphorylase b by covalent and allosteric regulation, respectively, is shown in Figure 3.16. For the interested reader, a biochemistry text [1] includes a more detailed account of how the phosphorylase reaction is regulated.

Although glycogen phosphorylase cleaves α 1-4 glycosidic bonds, it cannot hydrolyze α 1-6 bonds. The enzyme acts repetitively along linear portions of the glycogen molecule until it reaches a point four glucose residues from an α 1-6 branch point. Here the degradation process stops, resuming only after an enzyme called the debranching enzyme cleaves the α 1-6 bond at the branch point.

At times of heightened glycogenolytic activity, the formation of increased amounts of glucose 1-phosphate shifts the glucose phosphate isomerase reaction toward production of the 6-phosphate isomer. The glucose 6-phosphate can enter into the oxidative pathway for glucose, glycolysis, or become free glucose (in the liver or kidney). The conversion of glucose 6-phosphate to free glucose requires the action of glucose 6-phosphatase. This enzyme functions in liver and kidney cells but is not expressed in muscle cells or adipocytes. Therefore, free glucose can be formed from liver glycogen and transported through the

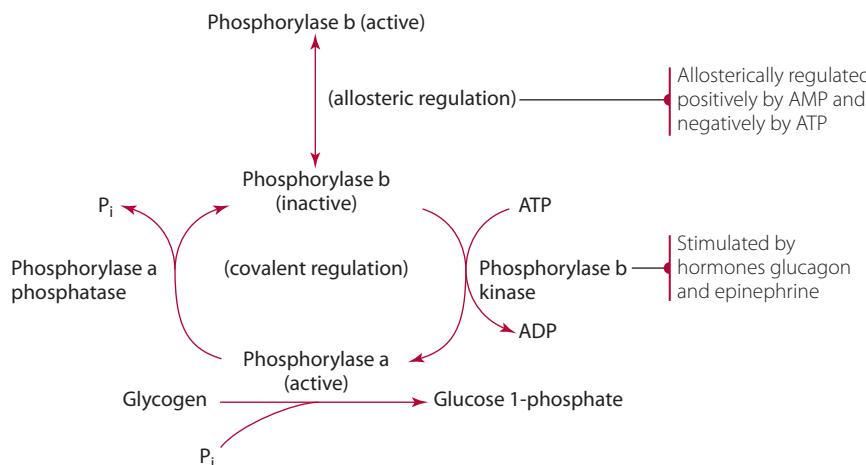


Figure 3.16 An overview of the regulation of glycogen phosphorylase. It is also regulated allosterically by AMP and ATP, which cause shifts in the equilibrium between inactive and active "b" forms. AMP positively modulates the enzyme by shifting the equilibrium toward its active "b" form. ATP inhibits the effect of AMP, thereby favoring the formation of the inactive "b" form. For more details, see the text under the description for reaction 1 of glycogenolysis.

bloodstream to other tissues for oxidation, but muscle glycogen cannot contribute to the blood glucose level. Thus, the liver (but not muscle) can control the concentration of glucose in the blood. Although muscle and, to some extent, adipose tissue have glycogen stores, these stores can be broken down to glucose only for use within the cell in which it is stored.

GLYCOLYSIS

Glycolysis is the pathway by which glucose is degraded into two units of pyruvate, a triose. From pyruvate, the metabolic course of the glucose depends largely on the availability of oxygen within the cell, and therefore the course is said to be either aerobic or anaerobic. Under anaerobic conditions—that is, in a situation of oxygen debt—pyruvate is converted to lactate. Under otherwise normal conditions, the conversion to lactate would occur mainly in times of strenuous exercise when the demand for oxygen by the working muscles exceeds that which is available. Lactate produced under anaerobic conditions can also diffuse from the muscle to the bloodstream and be carried to the liver for conversion to glucose. Under these anaerobic conditions, glycolysis releases a small amount of usable energy that can help sustain the muscles even in a state of oxygen debt. Providing this energy is the major function of the anaerobic pathway of glucose to lactate. Anaerobic glycolysis is the sole source of energy for erythrocytes, because the red blood cell does not contain mitochondria. The brain and GI tract produce much of their energy needs from glycolysis.

Under aerobic conditions, pyruvate can be transported into the mitochondria and participate in the TCA cycle, in which it becomes completely oxidized to CO_2 and H_2O . Complete oxidation is accompanied by the release of relatively large amounts of energy, much of which is salvaged as ATP by the mechanism of oxidative phosphorylation. The glycolytic enzymes function within the cytoplasmic matrix of the cell, but the enzymes catalyzing the TCA cycle

reactions are located within the mitochondrion. Therefore, pyruvate must enter the mitochondrion for complete oxidation. Glycolysis followed by TCA cycle activity (aerobic catabolism of glucose) demands an ample supply of oxygen, a condition that generally is met in normal, resting mammalian cells. In a normal, aerobic situation, complete oxidation of pyruvate generally occurs, with only a small amount of lactate being formed. The primary importance of glycolysis in energy metabolism, therefore, is in providing the initial sequence of reactions (to pyruvate) necessary for the complete oxidation of glucose by the TCA cycle, which supplies relatively large quantities of ATP.

In cells that lack mitochondria, such as the erythrocyte, the pathway of glycolysis is the sole provider of ATP by the mechanism of substrate-level phosphorylation of ADP, discussed later in this chapter. Nearly all cell types conduct glycolysis, but most of the energy derived from carbohydrates originates in liver, muscle, and adipose tissue, which together constitute a major portion of total body mass. The pathway of glycolysis, under both aerobic and anaerobic conditions, is summarized in Figure 3.17. Also indicated in the figure is the mode of entry of glucose from glycogenolysis, dietary fructose, and dietary galactose into the pathway for metabolism. Following are comments on selected reactions.

- ① The hexokinase/glucokinase reaction consumes 1 mol ATP/mol glucose. Hexokinase in muscle (but not glucokinase) is negatively regulated by the product of the reaction, glucose 6-phosphate. Glucokinase in liver (but not hexokinase) is induced by insulin.
- ② Glucose phosphate isomerase (also called hexose phosphate isomerase) catalyzes this interconversion of isomers—glucose 6-P to fructose 6-P.
- ③ The phosphofructokinase reaction, an important regulatory site, is modulated (by allosteric mechanisms) negatively by ATP and citrate and positively by AMP and ADP. In other words, when the cell has adequate energy

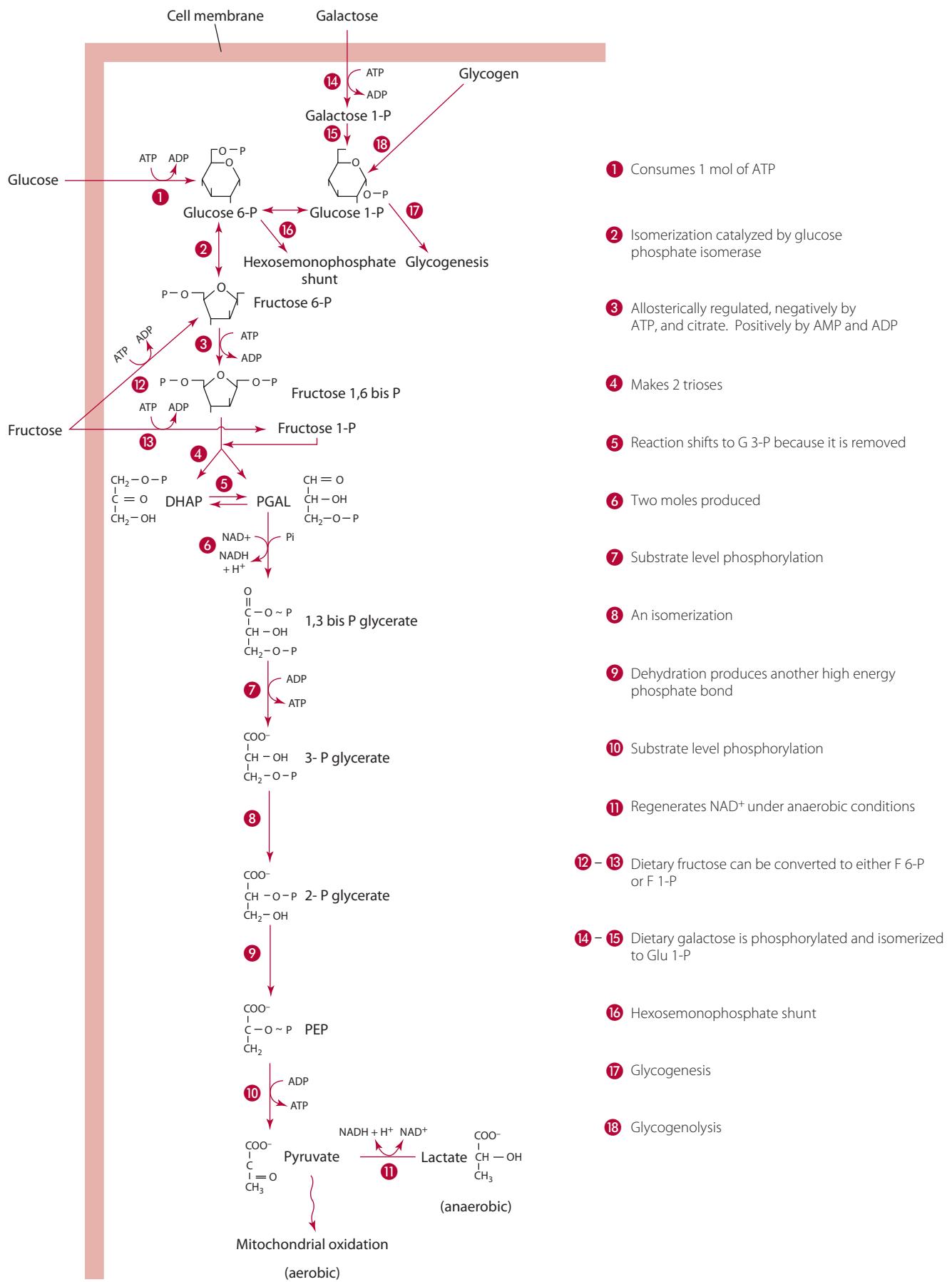


Figure 3.17 Glycolysis, indicating the mode of entry of glucose, fructose, glycogen, and galactose into glycolysis.

stores (ATP) the glycolytic pathway is inhibited and does not produce additional energy. Another ATP is consumed in the reaction. This important reaction is also regulated hormonally by glucagon (by induction), as described later in this chapter.

4 The aldolase reaction results in splitting a hexose (fructose) bisphosphate into two triose phosphates, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP). The prefix “bis” means two phosphates are present, on different carbons atoms. The prefix “di” means that the two phosphates are attached to each other and to a single carbon atom.

5 The isomers glyceraldehyde 3-phosphate and dihydroxyacetone phosphate are interconverted by the enzyme triosephosphate isomerase. In an isolated system, the equilibrium favors DHAP formation. In the cellular environment, however, it is shifted completely toward producing glyceraldehyde 3-phosphate, because this metabolite is continuously removed from the equilibrium by the subsequent reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase.

6 In this reaction, glyceraldehyde 3-phosphate is oxidized to a carboxylic acid, while inorganic phosphate is incorporated as a high-energy anhydride bond. The enzyme is glyceraldehyde 3-phosphate dehydrogenase, which uses NAD⁺ as its hydrogen-accepting cosubstrate. Under aerobic conditions, the NADH formed is reoxidized to NAD⁺ by O₂ through the electron transport chain in the mitochondria, as explained in the next section. The reason why O₂ is not necessary to sustain the reaction of converting glyceraldehyde 3-P to bis-P-glycerate is that under anaerobic conditions the NAD⁺ consumed is restored by a subsequent reaction converting pyruvate to lactate (see the reaction below).

7 This reaction, catalyzed by phosphoglycerate kinase, exemplifies a substrate-level phosphorylation of ADP. A more detailed review of substrate-level phosphorylation, by which ATP is formed from ADP by the transfer of a phosphate from a high-energy donor molecule, is covered below. Two ATPs are synthesized because glucose (a hexose) makes two trioses.

8 Phosphoglyceromutase catalyzes the transfer of the phosphate group from the number 3 carbon to the number 2 carbon of the glyceric acid.

9 Dehydration of 2-phosphoglycerate by the enzyme enolase introduces a double bond that imparts high energy to the phosphate bond.

10 Phosphoenolpyruvate (PEP) donates its phosphate group to ADP in a reaction catalyzed by pyruvate kinase. This is the second site of substrate-level phosphorylation of ADP in the glycolytic pathway to make two more ATPs.

11 The lactate dehydrogenase reaction transfers two hydrogens from NADH and H⁺ to pyruvate, reducing it to lactate. NAD⁺ is formed in the reaction and can replace the NAD⁺ consumed earlier under anaerobic conditions. This reaction is most active in situations of oxygen debt, as occurs in prolonged muscular activity. Under normal, aerobic conditions, pyruvate enters the mitochondrion for complete oxidation. A third important option available to pyruvate is its conversion to the amino acid alanine by transamination, a reaction by which pyruvate acquires an amino group from the amino acid glutamate (Chapter 6). The alternate pathways for pyruvate, together with the fact that pyruvate is also the product of the catabolism of various amino acids, makes pyruvate an important link between protein (amino acid) and carbohydrate metabolism.

12 and **13** These two reactions provide the means by which dietary fructose enters the glycolytic pathway. Fructose is an important factor in the average American diet, as nearly half the carbohydrate consumed is sucrose and high-fructose corn syrup, which is becoming more popular as a food sweetener. The phosphorylation of fructose to fructose 6-P function does not occur in tissues other than the liver. Fructose is directly phosphorylated by hexokinase to form fructose 6-phosphate. This is a relatively unimportant reaction, because the liver clears nearly all of the dietary fructose on the first pass. The hexokinase reaction is slow and occurs only in the presence of high levels of fructose. The phosphorylation of fructose to fructose 1-P is the major means by which fructose is converted to glycolysis metabolites. The phosphorylation occurs at the number 1 carbon and is catalyzed by fructokinase, an enzyme found only in liver cells. The fructose 1-phosphate subsequently is split by aldolase (designated aldolase B to distinguish it from the enzyme acting on fructose 1,6-bisphosphate), forming DHAP and glyceraldehyde. Glyceraldehyde can then be phosphorylated by glyceraldehyde kinase (or triokinase) at the expense of a second ATP to produce glyceraldehyde 3-phosphate. This reaction converts fructose to glycolytic intermediates, which can then follow the pathway to pyruvate formation and mitochondrial oxidation. Alternatively, the three-carbon intermediates can be used in the liver to produce free glucose by a reversal of the first part of the glycolytic pathway through the action of gluconeogenic enzymes. Glucose formation from fructose is particularly important if fructose provides the major source of carbohydrate in the diet. Because the phosphorylation of fructose is essentially the liver's responsibility, eating large amounts of fructose can deplete hepatocyte ATP and thus reduce the rate of various biosynthetic processes, such as protein synthesis.

14 Like glucose and fructose, galactose is first phosphorylated. The transfer of the phosphate from ATP is catalyzed by galactokinase, and the resulting phosphate ester is at the

number 1 carbon of the sugar. The major dietary source of galactose is lactose, from which the galactose is released by lactase during absorption.

15 Galactose 1-phosphate can be converted to glucose 1-phosphate through the intermediates uridine diphosphate (UDP)-galactose and uridine diphosphate (UDP)-glucose. The enzyme galactose 1-phosphate uridyl transferase transfers a uridyl phosphate residue from UDP-glucose to the galactose 1-phosphate, yielding glucose 1-phosphate and UDP-galactose. In a reaction catalyzed by epimerase, UDP-galactose can then be converted to UDP-glucose, in which form it can be converted to glucose 1-phosphate (by the uridyl transferase reaction already described) or be incorporated into glycogen by glycogen synthase, as described previously in the section “Glycogenesis.” It can also enter the glycolytic pathway as glucose 6-phosphate, made possible by the reaction series UDP-glucose glucose 1-phosphate glucose 6-phosphate. As glucose 6-phosphate, it can also be hydrolyzed to free glucose in liver cells (but not muscle).

16 This is the point where glucose 6-phosphate enters into a pathway called the hexosemonophosphate shunt (the pentose phosphate pathway), which is discussed later in this chapter.

17 This is the point of entry of glucose 1-phosphate into glycogenesis, the synthesis of glycogen.

18 By glycogenolysis, the glucose stored in glycogen can enter the glucose oxidative pathway (glycolysis).

SUBSTRATE-LEVEL PHOSPHORYLATION

The preceding discussion of glucose metabolism showed several enzyme-catalyzed reactions that resulted in phosphorylated products. Thinking of these products in terms of high-energy and low-energy phosphate bonds is easy, with ATP and other nucleoside triphosphates being high energy, and phosphate esters representing low energy. However, the wide range of ester energy, along with the fact that some phosphorylated compounds have even higher energy than ATP, complicates the high-energy/low-energy concept. Phosphoenolpyruvate and 1,3-diphosphoglycerate, which occur as intermediates in the metabolic pathway of glycolysis, and phosphocreatine, an important source of energy in muscle contraction, are examples of compounds having phosphate bond energies significantly higher than ATP. The structures of these compounds with the phosphate bonds highlighted are shown in Figure 3.18. Note that “high-energy” bonds are depicted as a wavy line (~, called a *tilde*), indicating that the free energy of hydrolysis is higher than it is for the more stable phosphate esters. Table 3.5 (on page 90) lists the standard free energy of hydrolysis of selected phosphate-containing compounds in both Kcal and kJ.

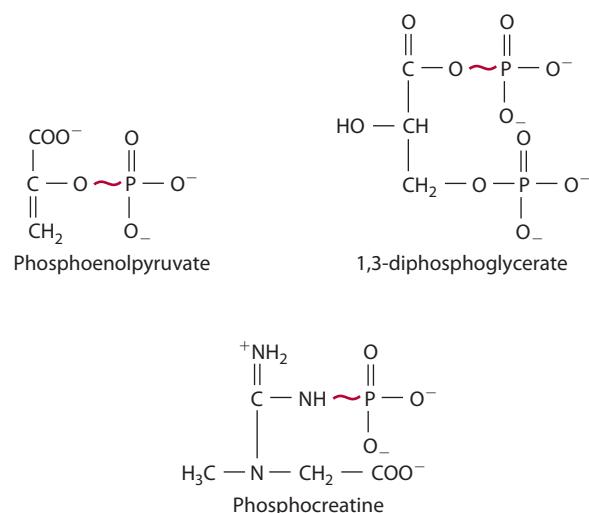


Figure 3.18 Examples of very high energy phosphate compounds. The phosphate bonds represented by the wavy lines contain more energy than the terminal phosphate bond of ATP, making it energetically possible to transfer these phosphate groups enzymatically to ADP.

THE TRICARBOXYLIC ACID CYCLE

The tricarboxylic acid cycle (TCA cycle), also called the Krebs cycle or the citric acid cycle, is at the forefront of energy metabolism in the body. It can be thought of as the common and final catabolic pathway, because products of carbohydrate, fat, and amino acids that enter the cycle can be completely oxidized to CO_2 and H_2O , with the accompanying release of energy. Over 90% of the energy released from food is estimated to occur as a result of TCA cycle oxidation. Not all the substances entering the cycle are totally oxidized, however. Some TCA cycle intermediates are used in the formation of glucose by the process of gluconeogenesis (discussed later), and some can be converted to certain amino acids by transamination (Chapter 6).

The TCA cycle is located within the matrix of the mitochondria. The high-energy output of the TCA cycle is attributed to mitochondrial electron transport, with oxidative phosphorylation being the source of ATP formation, as discussed later in this chapter. The oxidation reactions occurring in the cycle are actually dehydrogenations in which an enzyme catalyzes the removal of two hydrogens ($\frac{1}{2}\text{H}_2$) to an acceptor cosubstrate such as NAD^+ or FAD. Because both the enzymes of the cycle and the enzymes and electron carriers of electron transport are compartmentalized within the mitochondria, the reduced cosubstrates, NADH and FADH_2 , are readily reoxidized by O_2 through the electron transport chain, located in the mitochondrial inner membrane.

In addition to producing the reduced cosubstrates NADH and FADH_2 , which furnish the energy when they are oxidized during electron transport, the TCA cycle produces most of the carbon dioxide through decarboxylation

reactions. In terms of glucose metabolism, recall that two pyruvates are produced from one glucose molecule during cytoplasmic glycolysis. These pyruvates in turn are transported into the mitochondria, where decarboxylation leads to the formation of two acetyl CoA units and two molecules of CO_2 . The two carbons represented by the acetyl CoA are incorporated into citric acid and sequentially lost as two molecules of CO_2 through TCA cycle decarboxylations. Most of the CO_2 produced is exhaled through the lungs, although some is used in certain synthetic reactions called carboxylations.

TCA Pathway

The TCA cycle is shown in Figure 3.19. The acetyl CoA, which couples with oxaloacetate to begin the pathway, is formed from numerous sources, including the breakdown of fatty acids, glucose (through pyruvate), and certain amino acids. We consider the formation from pyruvate here, because pyruvate links cytoplasmic glycolysis to the TCA cycle. The first reaction shown in Figure 3.19 is called the pyruvate dehydrogenase reaction. The reaction is actually a complex one requiring a multienzyme system and various cofactors, with the enzymes and cofactors contained within an isolatable unit called the pyruvate dehydrogenase complex. The cofactors include coenzyme A (CoA), thiamin pyrophosphate (TPP), Mg^{2+} , NAD^+ , FAD, and lipoic acid. Four vitamins therefore are necessary for the activity of the complex: pantothenic acid (a component of CoA), thiamin, niacin, and riboflavin. The role of these vitamins and others as precursors of coenzymes is discussed in Chapter 9. The enzymes in the complex include pyruvate decarboxylase, dihydrolipoyl dehydrogenase, and dihydrolipoyl transacetylase. The net effect of the complex is decarboxylation and dehydrogenation of pyruvate, with NAD^+ serving as the terminal hydrogen acceptor. This reaction therefore yields energy, because the reoxidation of the NADH by electron transport produces approximately 3 mol of ATP by oxidative phosphorylation. The reaction is regulated allosterically negatively by acetyl CoA and by NADH, and positively by ADP and Ca^{2+} .

The condensation of acetyl CoA with oxaloacetate initiates the TCA cycle reactions. Following are comments on the reactions (Figure 3.19). The individual reactions will be discussed briefly:

- ① The formation of citrate from oxaloacetate and acetyl CoA is catalyzed by citrate synthase. The reaction is regulated negatively by ATP.
- ② The isomerization of citrate to isocitrate involves *cis*-aconitate as an intermediate. The isomerization, catalyzed by aconitase, involves dehydration followed by sterically reversed hydration, resulting in the repositioning of the $-\text{OH}$ group onto an adjacent carbon.
- ③ Catalyzed by the enzyme isocitrate dehydrogenase, this is the first of four dehydrogenation reactions within

the cycle. Energy is supplied from this reaction through the respiratory chain by the reoxidation of the NADH. Note that the first loss of CO_2 in the cycle occurs at this site. The CO_2 arises from the spontaneous decarboxylation of an intermediate compound, oxalosuccinate (not shown). The reaction is positively modulated by ADP and negatively modulated by ATP and NADH.

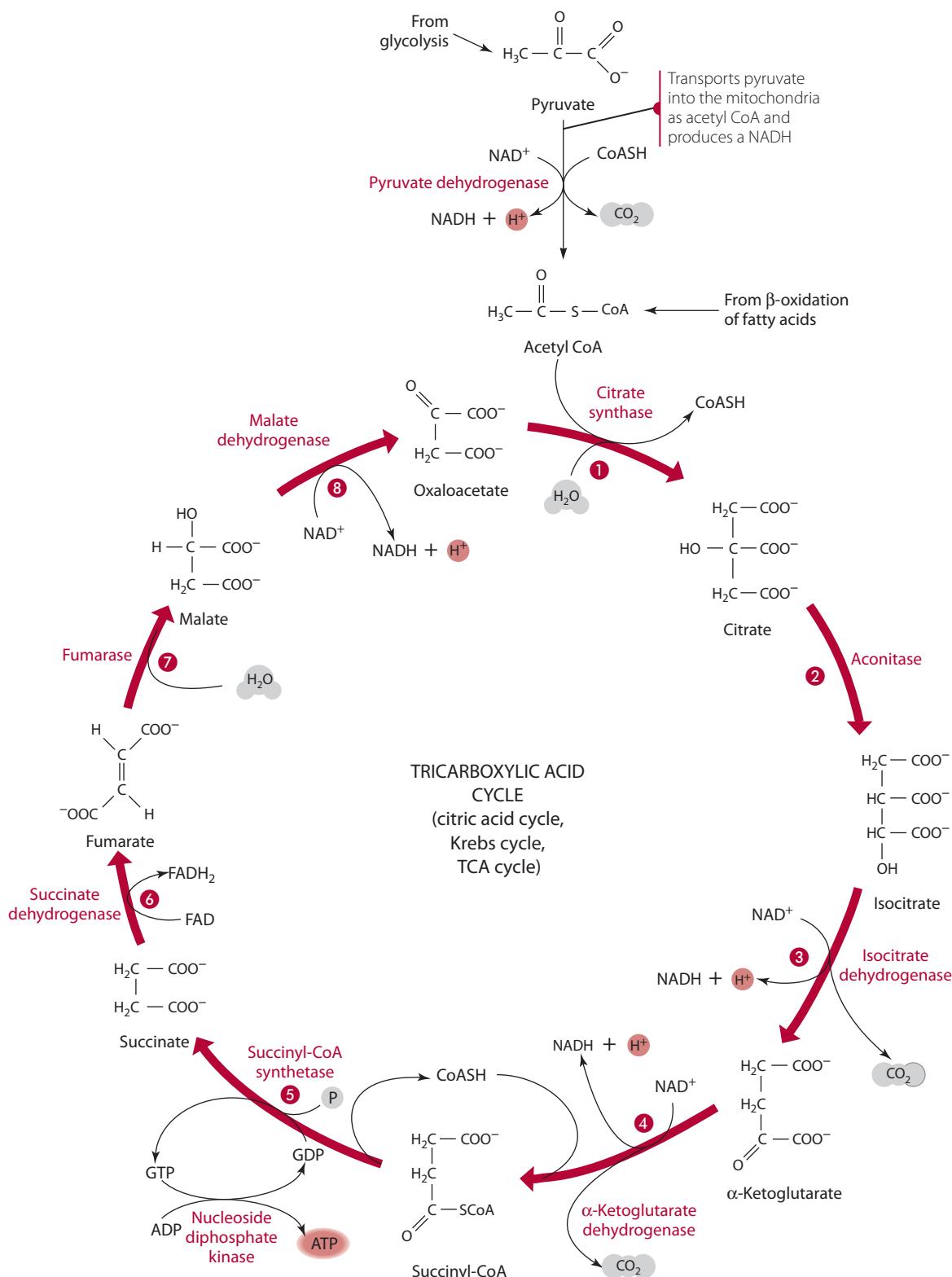
- ④ The decarboxylation and dehydrogenation of α -ketoglutarate is mechanistically identical to the pyruvate dehydrogenase complex reaction in its multienzyme-monicofactor requirement. In the reaction, called the α -ketoglutarate dehydrogenase reaction, NAD^+ serves as hydrogen acceptor, and a second carbon is lost as CO_2 . The pyruvate dehydrogenase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase reactions account for the loss of the three carbons from pyruvate as CO_2 .
- ⑤ Energy is conserved in the thioester bond of succinyl CoA. The hydrolysis of that bond by succinyl thiokinase releases sufficient energy to drive the phosphorylation of guanosine diphosphate (GDP) by inorganic phosphate. The resulting GTP is a high-energy phosphate anhydride compound like ATP. As such, GTP can serve as phosphate donor in certain phosphorylation reactions, for example, in reactions involved in gluconeogenesis or glycogenesis. GTP can transfer its γ -phosphate to ADP to form ATP.
- ⑥ The succinate dehydrogenase reaction uses FAD instead of NAD^+ as hydrogen acceptor. The FADH_2 is reoxidized by electron transport to O_2 , but only about two ATPs are formed by oxidative phosphorylation instead of three.
- ⑦ Fumarase incorporates the elements of H_2O across the double bond of fumarate to form malate.
- ⑧ The conversion of malate to oxaloacetate completes the cycle. NAD^+ acts as hydrogen acceptor in this dehydrogenation reaction, which is catalyzed by malate dehydrogenase. This reaction is the fourth site of reduced cosubstrate formation (3-NADH and 1-FADH₂) and thus results in additional energy release in the cycle.

ATPs Produced By Complete Glucose Oxidation

The complete oxidation of glucose to CO_2 and H_2O can be shown by this equation:



Complete oxidation is achieved by the combined reaction sequences of the glycolytic and TCA cycle pathways. By convention, it is assumed that 3 ATPS are formed by oxidative phosphorylation from NADH, and 2 ATPs are formed from FADH_2 . As discussed later in this chapter,



- ① Acetyl CoA adds 2 carbons to oxaloacetate to start the cycle.
- ② Isomerization takes place by removing H_2O and then adding it back.
- ③ A CO_2 is lost and a NADH is produced.
- ④ Another CO_2 is lost and another NADH is produced.
- ⑤ A substrate level phosphorylation.
- ⑥ FAD⁺ is reduced to form FADH₂.
- ⑦ Add H_2O across the double bond.
- ⑧ Third NADH produced in the TCA cycle. One FADH₂ and one NADH produced in the conversion of pyruvate to acetyl CoA.

Figure 3.19 The tricarboxylic acid (TCA) cycle.

the actual number of ATPs formed from NADH is closer to 2.5; for FADH₂, it is 1.5. For convenience and consistency we will continue to use the integers (3/2) for ATPs produced from NADH/FADH₂. Therefore, under aerobic conditions, the amount of released energy conserved as ATP is as follows:

- ① The glycolytic sequence, glucose → two pyruvates, produces four ATPs by substrate-level phosphorylation. However, two ATPs are used in the pathway, producing a net of two ATPs. (2 ATPs)
- ② The two NADHs formed in the glycolytic sequence at the glyceraldehyde 3-phosphate dehydrogenase reaction yield either four or six ATPs, depending on the shuttle system for moving the NADH-reducing equivalents into the mitochondria for reoxidation. The shuttle system is discussed later in this chapter. Generally, six ATPs are formed, because of the overall greater activity of the malate-aspartate shuttle system, as discussed later. (6 ATPs)
- ③ The intramitochondrial pyruvate dehydrogenase reaction yields 2 mol of NADH, one for each pyruvate oxidized, and therefore six additional ATPs by oxidative phosphorylation. (6 ATPs)
- ④ The oxidation of 1 mol of acetyl CoA in the TCA cycle yields 12 ATPs. These ATPs form at different sites, indicated by reaction number in Figure 3.19:

Reaction (3): 3 ATPs

Reaction (4): 3 ATPs

Reaction (5): 1 ATP (as GTP)

Reaction (6): 2 ATPs

Reaction (8): 3 ATPs

This process yields 12 ATPs × 2 mol of acetyl CoA per mol of glucose, which equals a total of 24 ATPs. (24 ATPs)

The total number of ATPs produced for the complete oxidation of 1 mol of glucose is therefore 38, equivalent to 262.8 kcal (1,100 kJ). This figure represents only about 40% of the total energy released by mitochondrial electron transport, because biological oxidation is only about 40% efficient. The remaining 60%, or approximately 394 kcal (1,650 kJ), is released as heat to maintain body temperature.

To summarize energy release from glycolysis in terms of ATP produced, substrate-level phosphorylation reactions result in a net of two ATPs. These two ATPs are all that is produced from anaerobic glycolysis. If the starting point of glycolysis is glycogen rather than free glucose, the hexokinase reaction is bypassed, and the energy yield is therefore increased by one ATP for glycolysis of glycogen glucose, under either aerobic or anaerobic conditions.

Under aerobic conditions, in contrast, additional ATPs are formed by oxidative phosphorylation. The cytoplasmic

NADH produced by glycolysis is not used in the (anaerobic) lactate dehydrogenase reaction but is reoxidized by the shuttle systems to NAD⁺ by electron transport and oxygen. The number of additional ATPs formed depends on which shuttle system is used to move the NADH hydrogens into the mitochondrion. If the malate-aspartate shuttle is in effect, six ATPs are produced, bringing the total to eight. In tissues using the glycerol 3-phosphate shuttle, just four ATPs are formed by oxidative phosphorylation, for a glycolytic total of six.

Acetyl CoA Oxidation and Tricarboxylic Acid Cycle Intermediates

A steady supply of four-carbon units is needed for the TCA cycle to oxidize all of the acetyl CoA produced to CO₂ and H₂O. In absence of four-carbon intermediates, ketoacidosis results. We will discuss this process briefly here and more extensively in Chapter 5. Acetyl CoA is produced by fatty acid oxidation and amino acid catabolism, as well as from the pyruvate derived from glycolysis (see Chapters 5 and 6). This increase in acetyl CoA leads to an imbalance between the amounts of acetyl CoA and oxaloacetate, which condense one-to-one stoichiometrically in the citrate synthase reaction. To keep the TCA cycle functioning, oxaloacetate and/or other TCA cycle intermediates that can form oxaloacetate must be replenished in the cycle. Such a mechanism does exist. Oxaloacetate, fumarate, succinyl CoA, and α-ketoglutarate can all be formed from certain amino acids, but the single most important mechanism for ensuring an ample supply of oxaloacetate is the reaction that forms oxaloacetate directly from pyruvate. This reaction, shown in Figure 3.20, is catalyzed by pyruvate carboxylase. The “uphill” incorporation of CO₂ is accomplished at the expense of ATP, and the reaction requires the participation of biotin (see Chapter 9). The conversion of pyruvate to oxaloacetate is called an anaplerotic (filling-up) process because of its role in restoring oxaloacetate to the cycle. Interestingly, pyruvate carboxylase is regulated positively by acetyl CoA, thereby accelerating oxaloacetate formation in response to increasing levels of acetyl CoA.

NADH in Anaerobic and Aerobic Glycolysis: The Shuttle Systems

Under anaerobic conditions, the NADH produced in the pathway of glycolysis (the glyceraldehyde 3-phosphate dehydrogenase reaction) cannot undergo reoxidation by mitochondrial electron transport because molecular oxygen is the ultimate oxidizing agent in that system. Instead, NADH is used in the lactate dehydrogenase reduction of pyruvate to lactate, thereby becoming reoxidized to NAD⁺ without involving oxygen. In this manner, NAD⁺ is restored to sustain the glyceraldehyde 3-phosphate dehydrogenase reaction, allowing the production of lactate to continue in the absence of oxygen.

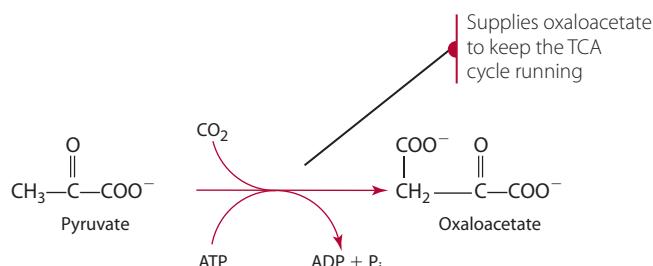


Figure 3.20 The reaction by which oxaloacetate is formed directly from pyruvate.

When glycolysis is operating aerobically, and the supply of oxygen is adequate to allow total oxidation of incoming glucose, lactic acid is not formed. Instead, pyruvate enters the mitochondrion, as does a carrier molecule of hydrogen atoms that were transferred to it from NADH. NADH cannot enter the mitochondrion directly. Rather, reducing equivalents in the form of carriers of hydrogen atoms ($\frac{1}{2}H_2$) removed from the NADH in the cytoplasm are shuttled across the mitochondrial membrane. Once in the mitochondrial matrix, the carriers are enzymatically dehydrogenated, and NAD^+ becomes reduced to NADH. NADH can then become oxidized by electron transport and consequently can generate about three ATPs per mole of NADH by oxidative phosphorylation. In this manner, six ATPs are formed aerobically per mole of glucose. The result of the shuttle system is therefore equivalent to a transfer of NADH from the cytoplasm into the mitochondrion, although the transfer does not occur directly.

Shuttle substances that transport the hydrogens removed from cytoplasmic NADH into the mitochondrion are glycerol 3-phosphate and malate. Figure 3.21 illustrates how the glycerol 3-phosphate shuttle systems function in the reoxidation of cytoplasmic NADH. The shuttle systems are specific to certain tissues. The more active malate-aspartate shuttle functions in the liver, kidney, and heart, whereas the glycerol 3-phosphate shuttle functions in the brain and skeletal muscle.

Glycerol 3-Phosphate Shuttle System Glycerophosphate produced by glycolysis is oxidized by two different glycerocephosphate dehydrogenases, one in the cytoplasm and the other on the outer face of the inner mitochondrial membrane. The glycerol 3-phosphate shuttle, in contrast to the malate-aspartate shuttle, leads to only two ATPs per mole of cytosol-produced NADH because the intramitochondrial reoxidation of glycerol 3-phosphate is catalyzed by glycerol phosphate dehydrogenase, which uses FAD instead of NAD^+ as hydrogen acceptor. Therefore, if the glycerol 3-phosphate shuttle is in effect, only four ATPs are formed aerobically per mole of glucose by oxidative phosphorylation (Figure 3.21).

Malate-Aspartate Shuttle System The most active shuttle compound, malate, is freely permeable to the inner

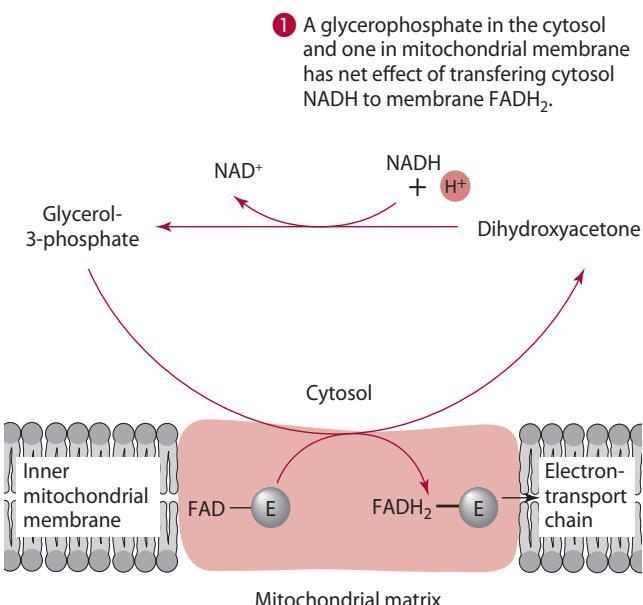


Figure 3.21 Glycerol-3-phosphate shuttle

mitochondrial membrane. The malate is oxidized by the enzyme malate dehydrogenase to oxaloacetic acid in the matrix of the mitochondria, producing NADH as a cofactor, which generates about three ATPs per mole. The oxaloacetic acid undergoes transamination by aspartate aminotransferase to form aspartate, which is freely permeable to the inner membrane and can move back out into the cytosol. The effect is that NADH moves into the mitochondria, even though the inner mitochondrial membrane is impermeable to it (Figure 3.22).

FORMATION OF ATP

In the preceding discussion, it was shown that certain molecules can be activated by phosphate group transfer from ATP. The phosphorylation itself is an endothermic reaction but is made possible by the highly exothermic hydrolysis of the terminal phosphate of ATP. For these processes to continue in the body, the ADP produced by the reaction must be reconverted to ATP to maintain the homeostatic concentration of cellular ATP. How is this conversion accomplished, considering the large amount of energy ($\Delta G^0 = +7,300 \text{ cal/mol}$) ($+35.7 \text{ kJ/mol}$) required for the reaction?

Obviously, outside sources of considerable energy must be linked to the phosphorylation of ADP. Actually, two such mechanisms function in this respect, substrate-level phosphorylation and oxidative phosphorylation. Substrate-level phosphorylation has already been discussed, but certain aspects are highlighted here. From the standpoint of

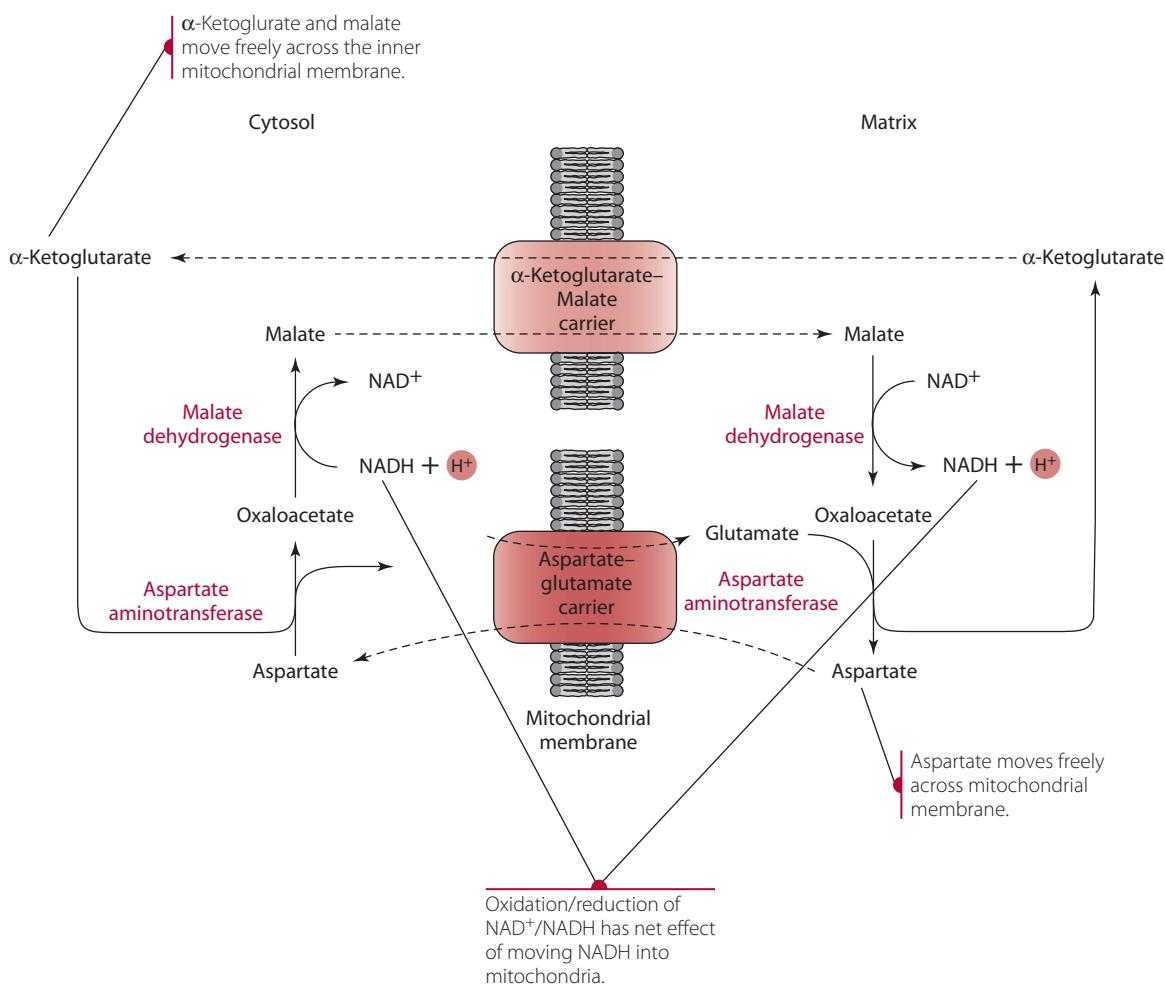


Figure 3.22 Malate-aspartate shuttle.

the amount of ATP produced, oxidative phosphorylation is decidedly the more important of the two mechanisms.

As shown in Table 3.5, phosphorylated molecules have a wide range of free energies of hydrolysis of their phosphate groups. Many of them release less energy than ATP, but some release more. The ΔG° of hydrolysis of the compounds listed in Table 3.5, called the phosphate group transfer potential, is a measure of the compounds' capacities to donate phosphate groups to other substances. The more negative the transfer potential, the more potent the phosphate-donating power. Therefore, a compound that

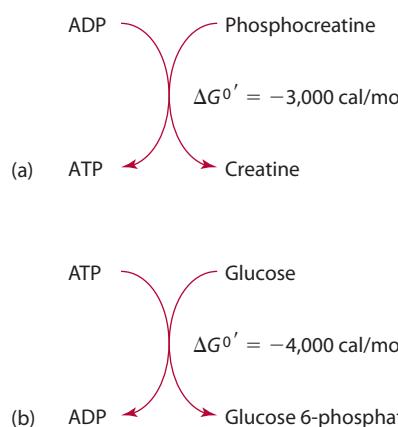
releases more energy on hydrolysis of its phosphate can transfer that phosphate to an acceptor molecule having a relatively more positive transfer potential. For this transfer to occur in actuality, however, there must be a specific enzyme to catalyze the transfer. A phosphate group can be enzymatically transferred from ATP to glucose, a transfer that can be predicted from Table 3.5. It can also be predicted from Table 3.5 that compounds with a more negative phosphate group transfer potential than ATP can transfer phosphate to ADP, forming ATP. This kind of reaction does, in fact, occur in metabolism. The phosphorylation of ADP by phosphocreatine, for example, represents an important mode for ATP formation in muscle, and the reaction exemplifies a substrate-level phosphorylation (Figure 3.23).

Table 3.5 Free Energy of Hydrolysis of Some Phosphorylated Compounds

Compound	$\Delta G^{\circ}(\text{cal})$	$\Delta G^{\circ}(\text{kJ})$
Phosphoenolpyruvate	-14,800	-62.2
1,3-diphosphoglycerate	-11,800	-49.6
Phosphocreatine	-10,300	-43.3
ATP	-7,300	-35.7
Glucose 1-phosphate	-5,000	-21.0
Adenosine monophosphate (AMP)	-3,400	-9.2
Glucose 6-phosphate	-3,300	-13.9

Biological Oxidation and the Electron Transport Chain

The major means by which ATP is formed from ADP is through the mechanism of oxidative phosphorylation. This process is discussed in detail here because ATP is the major supplier of energy from carbohydrates. Oxidative phosphorylation is also the major supplier of energy from lipids and amino acids. The required energy to form ATP

**Figure 3.23**

(a) Example of high-energy phosphate bond being transferred from high-energy compound phosphocreatine to form ATP. (b) The transfer of the high-energy phosphate bond to a compound that becomes activated, allowing it to enter into the glycolytic pathway.

is tapped from a pool of energy generated by the flow of electrons from substrate molecules undergoing oxidation and the translocation of protons (H^+). The electrons are then passed through a series of intermediate compounds and ultimately to molecular oxygen, which becomes reduced to H_2O in the process. The compounds participating in this sequential reduction-oxidation constitute the respiratory chain, so named because the electron transfer is linked to the uptake of O_2 , which is made available to the tissues by respiration. *Electron transport chain*, a more commonly used alternate term, is used instead throughout this text. The chain functions within the cell mitochondria. These organelles are often called the power plants of the cell, because of the large amount of energy liberated by electron transport. This energy assumes the form of heat to maintain body temperature and also is used to form ATP from ADP and P_i . Therefore, the term *oxidative phosphorylation* is a descriptive blend of two processes operating simultaneously:

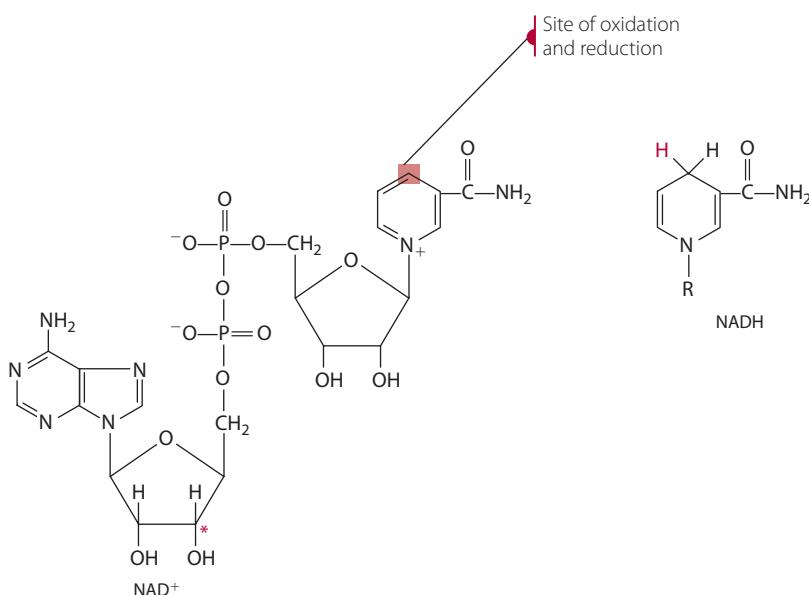
- the oxidation of a metabolite by O_2 through electron transport
- the phosphorylation of ADP

The processes of cellular oxidation of nutrients, electron transport, and oxidative phosphorylation perform a unified function and should be thought of together. They are considered next in more detail.

Oxidation of the energy nutrients from food (carbohydrates, protein, lipids, and alcohol) is what releases their inherent chemical energy and makes it available to the body either as heat or as ATP. This section discusses the nature of the oxidative processes that can occur and that are directly involved in energy production.

Cellular oxidation of a compound can occur by several different reactions: the addition of oxygen, the removal of electrons, and the removal of hydrogens (atoms of H or $\frac{1}{2}H_2$, not hydrogen ions). All these reactions are catalyzed by enzymes collectively termed oxidoreductases. Among these, the **dehydrogenases**, which remove hydrogens and electrons from nutrient metabolites, are particularly important in energy transformation. The hydrogens and electrons removed from metabolites by dehydrogenase reactions pass along the components of the electron transport chain and cause the release of large amounts of energy. In reactions in which oxygen is incorporated into a compound or hydrogens are removed by other than dehydrogenases, the electron transport chain is not called into play, and no energy is released. Such reactions are catalyzed by a subgroup of oxidoreductase enzymes generally called oxidases; these are not considered further in this section.

The hydrogen ions and electrons (which together are equivalent to $\frac{1}{2}H_2$) removed from a substrate molecule by a dehydrogenase enzyme are transferred to a cosubstrate, such as the vitamin-derived nicotinamide adenine dinucleotide (NAD^+) or flavin mononucleotide (FMN). The structures of these cosubstrates, with both the oxidized and reduced forms, are shown Figures 3.24 and 3.25. An example of energy release with oxidation is the oxidation

**Figure 3.24** Nicotinamide adenine dinucleotide (NAD^+) and its reduced form (NADH).

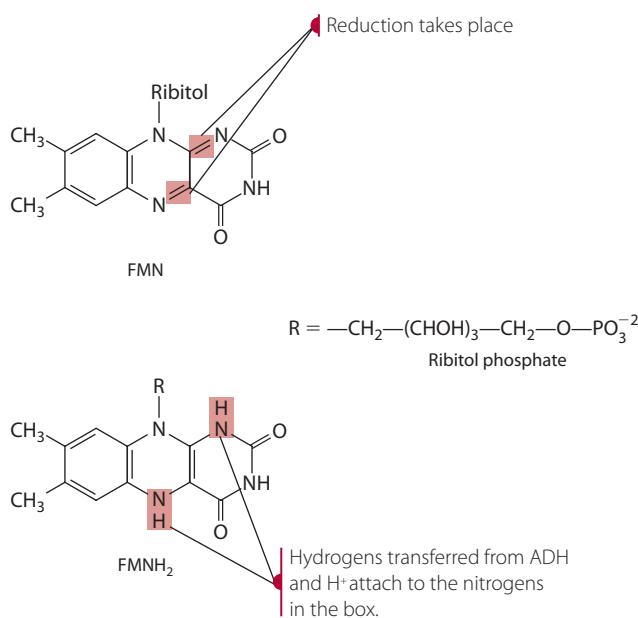


Figure 3.25 Flavin mononucleotide (FMN) and its reduced form (FMNH₂).

of the fatty acid palmitate has already been discussed and is shown in Figure 1.14. These reactions occur in the mitochondria. The sequential arrangement of reactions in the electron transport chain is shown in Figure 3.26. Dashed lines outline the four complexes. Either NADH or FADH₂ are the initial hydrogen acceptor for the electron transport chain. The hydrogens and electrons are then enzymatically transferred through the electron transport chain components and eventually to molecular oxygen, which becomes reduced to H₂O.

Anatomical Site for Oxidative Phosphorylation

The structure of the mitochondrion is described in Figures 1.6 and 1.7. Refer to Chapter 1 for a description of the outer membrane, which is permeable to most molecules smaller than 10 kilodaltons, and the inner membrane, which has very limited permeability. Remember that the enzymes of the TCA cycle, except for one, and of fatty acid oxidation discussed in Chapter 5, are located in the matrix of the mitochondria. The one enzyme of the TCA cycle that is an integral part of the inner membrane is succinate dehydrogenase, the importance of which is discussed later in this chapter. The translocation of H⁺ (protons) from within the matrix to the inner membrane space (the space between the cristae and outer membrane) provides much of the energy that drives the phosphorylation of ADP to make ATP. Note the respiratory stalks on the inner membrane (Figure 1.6), which also play an important role in the mechanism of oxidative phosphorylation. The electron transport chain starts with NADH and FADH₂, whether it is shuttled in from the cytoplasm, as discussed previously, or produced within the mitochondria.

Components of the Oxidative Phosphorylation Chain

Substrate phosphorylation of ADP to form ATP in the glycolytic pathway and the TCA cycle has already been discussed. These pathways also produce NADH and FADH₂, and their shuttling into the mitochondria has already been discussed. Most of the ATP produced from the macronutrients in food is produced by phosphorylation coupled to the electron transport chain. Figure 3.24 shows the oxidation and reduction that occurs in electron

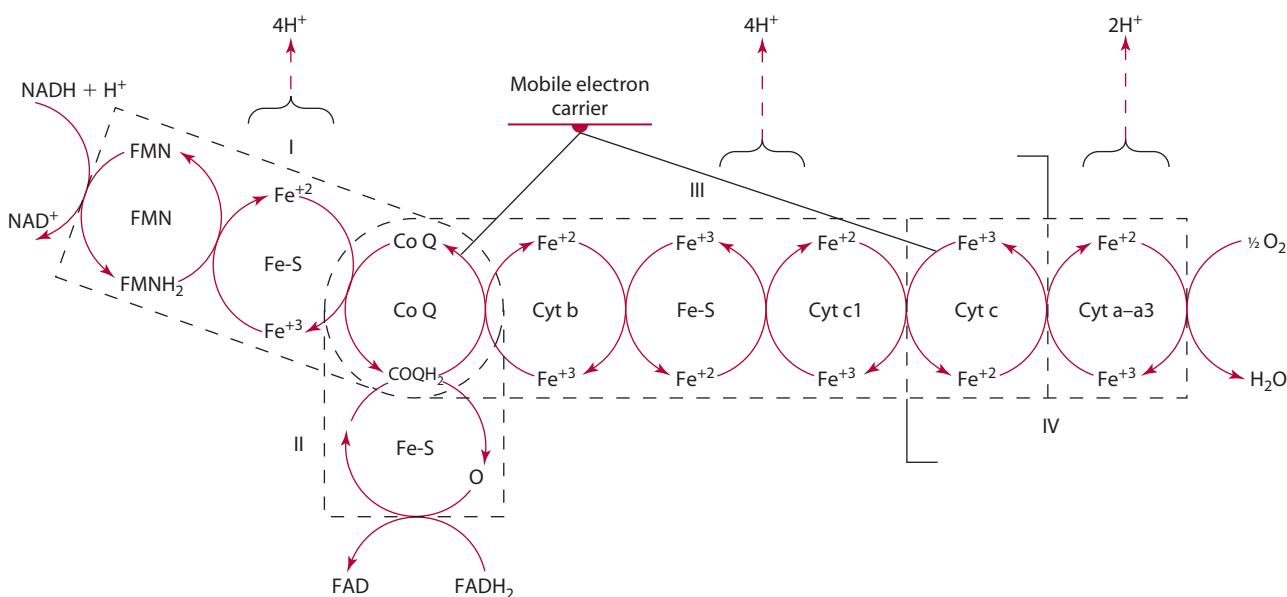


Figure 3.26 The sequential arrangement of the components of the electron transport chain, showing its division into four complexes, I, II, III, and IV. Coenzyme Q (ubiquinone) is shared by Complexes I, II, and III. Cyt c is shared by Complexes III and IV.

transport in the mitochondria. However, this view is too simplistic. The reactions actually take place in complexes of associated proteins and enzymes. The electron transport chain is made of four distinct complexes, which can be isolated and purified. Complex I NADH-coenzyme Q reductase accepts electrons from NADH and is the link with glycolysis, the TCA cycle, and fatty acid oxidation. Complex II succinate CoQ dehydrogenase includes the membrane-bound succinate dehydrogenase that is part of the TCA cycle. Both Complex I and II produce CoQH_2 . Reduced coenzyme Q is the substrate for Complex III, coenzyme Q-cytochrome c reductase. Complex IV is cytochrome oxidase. It is responsible for reducing molecular oxygen to form H_2O . A schematic depiction of the complexes is shown in Figure 3.27. The complexes work independently and are connected by mobile acceptors of electrons, coenzyme Q and cytochrome c. Each complex is discussed briefly here. For a more detailed explanation consult a general biochemistry textbook [1].

Complex I NADH-Coenzyme Q Oxidoreductase Complex I transfers a pair of electrons from NADH to coenzyme Q. The structures of the oxidized and reduced forms of coenzyme Q are shown in Figure 3.28. The enzyme is also called NADH dehydrogenase. The complex is made of several polypeptide chains, a molecule of FMN and several Fe-S clusters, along with additional iron molecules. The iron molecules bind with the sulfur-containing amino acid cysteine. The iron transfers one electron at a time cycling between $\text{Fe}^{+2}/\text{Fe}^{+3}$. The result of the multi-step reaction is the transfer of electrons and hydrogen from NADH to form reduced coenzyme Q and the transfer of hydrogen ions from the matrix side of the inner mitochondrial membrane to the cytosolic side of the inner membrane. The importance of the buildup of hydrogen ions in the inner membrane space is discussed in the following sections. The oxidation of NADH through the electron transport chain results in the synthesis of approximately 3 ATP molecules.

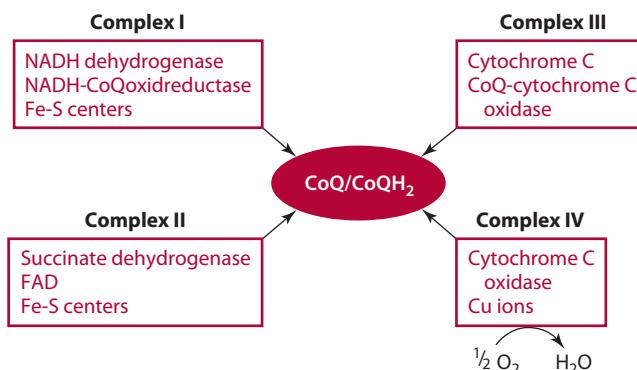


Figure 3.27 Schematic of electron transport modules connecting through coenzyme Q.

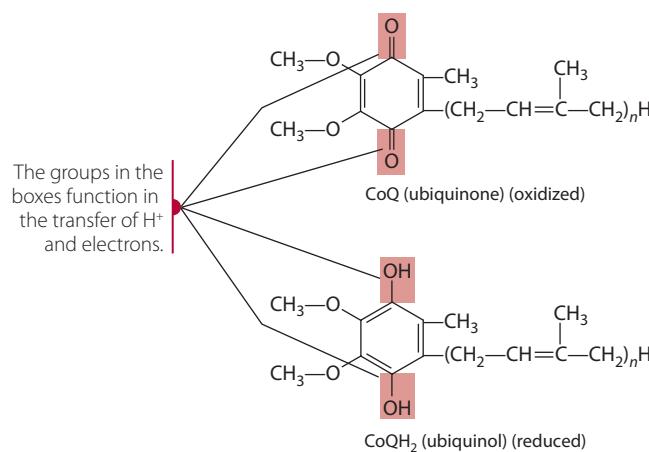


Figure 3.28 Oxidized and reduced forms of coenzyme Q, or ubiquinone. The subscript n indicates the number of isoprenoid units in the side chain (most commonly 10).

Complex II Complex II contains succinate dehydrogenase, which is the only TCA cycle enzyme that is an integral part of the inner mitochondrial membrane. Beside the succinate dehydrogenase, complex II contains a FAD protein and Fe-S clusters (similar to those discussed previously). When succinate is converted to fumarate in the TCA cycle FAD is reduced to FADH_2 . The FADH_2 is oxidized with one electron transfers through the Fe-S centers to reduce coenzyme Q to coenzyme QH_2 . The oxidation of FADH_2 through the electron transport chain results in the formation of approximately 2 molecules of ATP.

Complex III Coenzyme Q-Cytochrome C Oxidoreductase Reduced coenzyme Q passes its electrons to cytochrome c in the third complex of the electron transport chain in a pathway known as the Q cycle. The complex contains three different cytochromes and Fe-S protein. The cytochromes contain heme molecules with an iron molecule in the center. The iron in the center of the cytochromes is oxidized and reduced as electrons flow through. As electrons pass through the Q cycle, protons are transported across the inner mitochondrial membrane. Complex III takes up two protons on the matrix side of the inner membrane and releases four protons into the inner membrane space for each pair of electrons passing through the complex. Like coenzyme Q, cytochrome c is a mobile carrier. This characteristic means that cytochrome c is able to migrate along the membrane. Cytochrome c associates loosely with inner mitochondrial membrane on the matrix side of the membrane. It can then pass its electrons on to cytochrome c oxidase in complex IV, which is discussed next.

Complex IV Complex IV is also called cytochrome c oxidase. It accepts electrons from cytochrome c and reduces oxygen to form water. This reaction is the final one in the

oxidation of the energy-providing nutrients (carbohydrate, fat, protein, and alcohol) to produce usable chemical energy in the form of ATP. The structure of cytochrome c oxidase is known; it is made up of multiple subunits. Some of the subunits are encoded from nuclear DNA and some from mitochondrial DNA. These later proteins contain the iron and copper. These metal ions cycle between their oxidized (Fe^{+3} , Cu^{+2}) and reduced (Fe^{+2} , Cu^{+1}) states. Cytochrome c oxidase also contains two cytochromes, cytochrome a and cytochrome a_3 , which contain different heme moieties.

Electron transport can carry on without phosphorylation, but the phosphorylation of ADP to form ATP is dependent upon electron transport. This process is discussed in the next section. A schematic of the inner mitochondrial membrane showing the four complexes of the electron transport chain is shown in Figure 3.29. The free energy change at various sites within the electron transport chain is shown in Table 3.6.

Phosphorylation of ADP to Form ATP

The intimate association of energy release with oxidation is exemplified by the oxidation of the fatty acid palmitate shown in Figure 1.14. These reactions occur in the mitochondria. We have already examined the difference in $E_{0'}$ values between the NAD^+ - NADH to demonstrate

Table 3.6 Free Energy Changes at Various Sites Within the Electron Transport Chain Showing Phosphorylation Sites

Reaction	ΔG° (cal/mol)	ADP Phosphorylation
$\text{NAD}^+ \longrightarrow \text{FMN}$	-922	
$\text{FMN} \longrightarrow \text{CoQ}$	-15,682	$\text{ADP} + \text{P} \longrightarrow \text{ATP}$
$\text{CoQ} \longrightarrow \text{cyt b}$	-1,380	
$\text{cyt b} \longrightarrow \text{cyt c1}$	-7,380	$\text{ADP} + \text{P} \longrightarrow \text{ATP}$
$\text{cyt c1} \longrightarrow \text{cyt c}$	-922	
$\text{cyt c} \longrightarrow \text{cyt a}$	-1,845	
$\text{cyt a} \longrightarrow \frac{1}{2}\text{O}_2$	-24,450	$\text{ADP} + \text{P} \longrightarrow \text{ATP}$

sufficient energy is available to support phosphorylation of ADP. The overall change in free energy across electron transport chain:

$$\begin{aligned}\Delta E_{0'} &= E_{0'}(\text{O}_2) - E_{0'}(\text{NAD}^+) \\ &= 0.82 - (-0.32) = 1.14 \text{ volts}\end{aligned}$$

Then

$$\Delta G = 2(23,062)(1.14) = -52,581 \text{ cal/mol}$$

Under the standard conditions, 21,900 cal/mol ($3 \times 7,300$) of this total energy is conserved for future use as ATP, while the remaining 30,681 calories, representing close to 60% of the total, is released in the form of heat necessary to help maintain a normal body temperature.

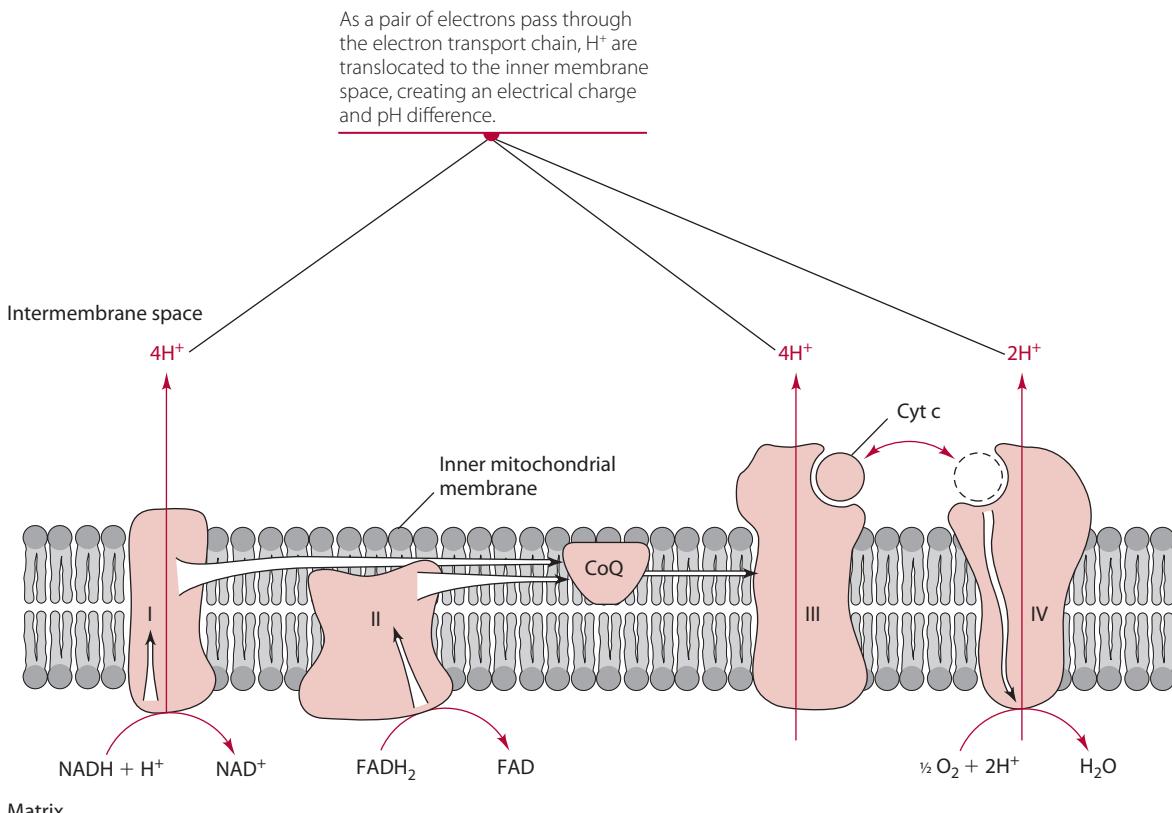


Figure 3.29 The spatial orientation of the complexes of the electron transport chain in the inner membrane of the mitochondrion.

The previous discussion focused on the translocation of hydrogen ions from the matrix to the inner mitochondrial space. This translocation of hydrogen ions requires energy but in return creates a pool of potential energy that can be used to synthesize ATP. The generally accepted mechanism for the synthesis of ATP was first proposed by Peter Mitchell in 1961. He proposed that the energy stored in the difference in the concentration of H⁺ between the matrix of the mitochondria and the inner mitochondrial space was the driving force for coupled ATP formation. This proposal was called the chemiosmotic hypothesis. A recent review presents current research about proton translocation [20]. We will examine its main points to support our understanding of the coupling of phosphorylation with the electron transport chain.

Translocation of H⁺

These energy relationships become important in understanding energy balance discussed in Chapter 8. This area of research has been intense. Many components of the hypothesis are supported by evidence. To determine if the energy in the gradient is sufficient, we must examine the number of hydrogen ions translocated at each complex. Direct measurements have been difficult and not everyone agrees. The consensus is for every 2 electrons that pass through Complex I (NADH dehydrogenase) 4 H⁺ are translocated, for each pair of electrons passing through Complex III 4 H⁺ are translocated, and for Complex IV an additional 2 H⁺ are translocated by each pair of electrons passing through the complex. No hydrogen ions are translocated in Complex II. This means that for every NADH oxidized to water, a total of 10 hydrogen ions are translocated from the matrix to the intermembrane space. The electrical charge across the inner membrane changes because of the positive hydrogen ions in the intermembrane space, a difference estimated to be approximately 0.18 volts. It is also assumed that the pH difference between the mitochondrial matrix and the inner membrane is 1 unit. Using these assumptions the free energy available is -94.49 kcal/mol (-23.3 kJ/mol). This is the potential free energy available to move protons back into the matrix of the mitochondria and at the same time couple phosphorylation of ADP to ATP. Paul Boyer and John Walker shared the 1997 Nobel Prize for chemistry for their work on ATP synthase. A review of Paul Boyer's research on ATP synthase sums up several decades of work [21].

ATP Synthase

The disparity in both the hydrogen ion concentration and electrical charge on either side of the inner membrane of the mitochondria has already been discussed. ATP synthase is made up of five polypeptide chains. ATP synthase also contains three hydrophobic subunits that form

a channel through the membrane which move protons and drive ATP synthesis. The channels are constructed from protein aggregates and exist as two distinct sectors designated F₁ and F₀. F₁ is the catalytic sector and resides on the matrix side of the membrane. F₀ is the membrane sector and is involved primarily with proton translocation (Figure 3.30). Together, these components make up what is called the F₀F₁ ATPase (or ATP synthase) aggregate, also referred to as Complex V. The return flow of protons furnishes the energy necessary for the synthesis of ATP from ADP and P_i. The proton flow is directed by the F₀ sector to the F₁ headpiece, which has binding sites for ADP and P_i. One oxygen atom of the inorganic phosphate is believed to react with two of the energetic protons, eliminating H₂O from the molecule. The precise mechanism of phosphorylation is complicated and involves the spatial movement of the complex protein.

Recall that the energy available from the oxidation of NADH (+H⁺) is sufficient to produce about 3 ATPs by the phosphorylation of 3 ADPs. Current research based on the number of protons translocated suggests that the theoretical number is closer to 2.5 ATPs produced for each NADH. This number accounts for the substrates for ATPase—ADP and P_i—that must be actively transported into the mitochondrion from the cytoplasm, as well as the product, ATP, that must be actively transported out. The estimated number of ATPs formed from NADH oxidation is therefore more realistically 2.5 rather than 3, and from FADH₂ the number is 1.5 rather than 2. Disagreement still exists about the correct number of ATPs formed. Convention still uses the whole number stoichiometry for most purposes. Throughout this text, we adhere to the convention of ascribing the values 3 and 2 to the number of moles of ATP produced from the oxidation of molar amounts of NADH and FADH₂, respectively. The net yield of ATP from glucose oxidation depends on the shuttle used to move the NADH into the mitochondria. If the glycerol 2-phosphate shuttle is used, FADH₂ is produced in the matrix of the mitochondria, and 2 ATPs are formed. If malate-aspartate shuttle is used, a NADH is produced in the matrix of the mitochondria, and 3 ATPs are produced.

The previous discussion of the conversion of the chemical energy of carbohydrates to form ATP is an integral part of carbohydrate metabolism. The next sections cover other aspects of carbohydrate metabolism. Comprehensive reviews of electron transport, oxidative phosphorylation, and proton translocation are available to the interested reader [22–24].

THE HEXOSEMONOPHOSPHATE SHUNT (PENTOSE PHOSPHATE PATHWAY)

The hexosemonophosphate shunt is one of the pathways shown in Figure 3.31 that is available to glucose. The purpose

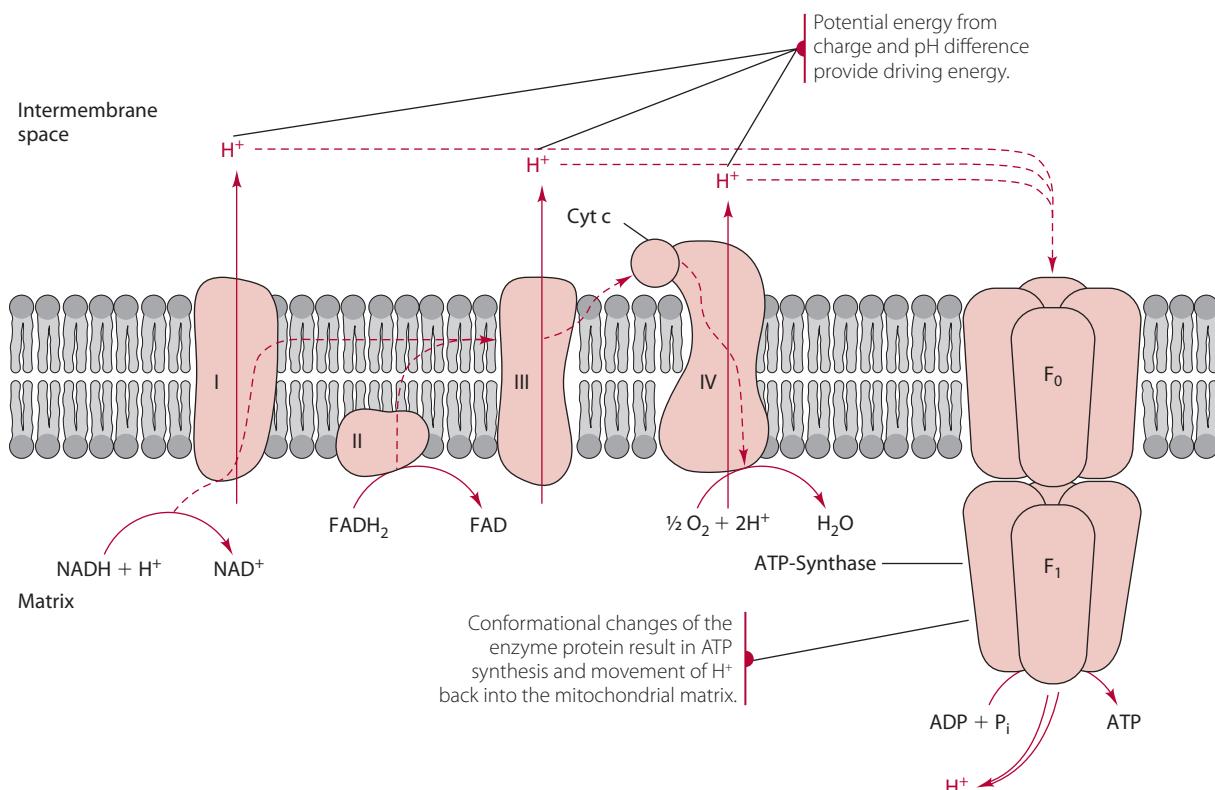


Figure 3.30 An illustration of oxidative phosphorylation coupled with ATP synthase. Energy from electron transport pumps protons into the intermembrane space from the matrix against a concentration gradient. The passive diffusion of protons back into the matrix through the F_0F_1 ATP-synthase aggregate furnishes the energy to synthesize ATP from ADP and inorganic phosphate. For more details, see the text.

of a metabolic shunt is to generate important intermediates not produced in other pathways. The hexosemonophosphate shunt has two very important products:

- pentose phosphates, necessary for the synthesis of the nucleic acids found in DNA and RNA and for other nucleotides (Figure 3.4)
- the reduced cosubstrate NADPH, used for important metabolic functions, including the biosynthesis of fatty acids (Chapter 5), the maintenance of reducing substrates in red blood cells necessary to ensure the functional integrity of the cells, and drug metabolism in the liver

The hexosemonophosphate shunt begins by oxidizing glucose 6-phosphate in two consecutive dehydrogenase reactions catalyzed by glucose 6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconate dehydrogenase (6-PGD). Both reactions require $NADP^+$ as cosubstrate, accounting for the formation of NADPH as a reduction product. The first reaction, (G-6-PD) is irreversible and highly regulated. It is strongly inhibited by the cosubstrate NADPH. Pentose phosphate formation is achieved by the decarboxylation of 6-phosphogluconate to form the pentose phosphate, ribulose 5-phosphate, which in turn is isomerized to its aldose isomer, ribose 5-phosphate.

Pentose phosphates can subsequently be “recycled” back to hexose phosphates through the transketolase and transaldolase reactions illustrated in Figure 3.31. This recycling of pentose phosphates to hexose phosphates therefore does not produce pentoses, but it does assure generous production of NADPH as the cycle repeats.

The cells of some tissues have a high demand for NADPH, particularly those that are active in the synthesis of fatty acids, such as the mammary gland, adipose tissue, the adrenal cortex, and the liver. These tissues predictably engage the entire pathway, recycling pentose phosphates back to glucose 6-phosphate to repeat the cycle and assure an ample supply of NADPH. The pathway reactions that include the dehydrogenase reactions and therefore the formation of NADPH from $NADP^+$ are called the oxidative reactions of the pathway. This segment of the pathway is illustrated on the left in Figure 3.31. The re-formation of glucose 6-phosphate from the pentose phosphates, through reactions catalyzed by transketolase, transaldolase, and hexose phosphate isomerase, are called the nonoxidative reactions of the pathway and are shown on the right in Figure 3.31. Transketolase and transaldolase enzymes catalyze complex reactions in which three-, four-, five-, six-, and seven-carbon phosphate sugars are interconverted. These reactions are detailed in most comprehensive biochemistry texts [1].

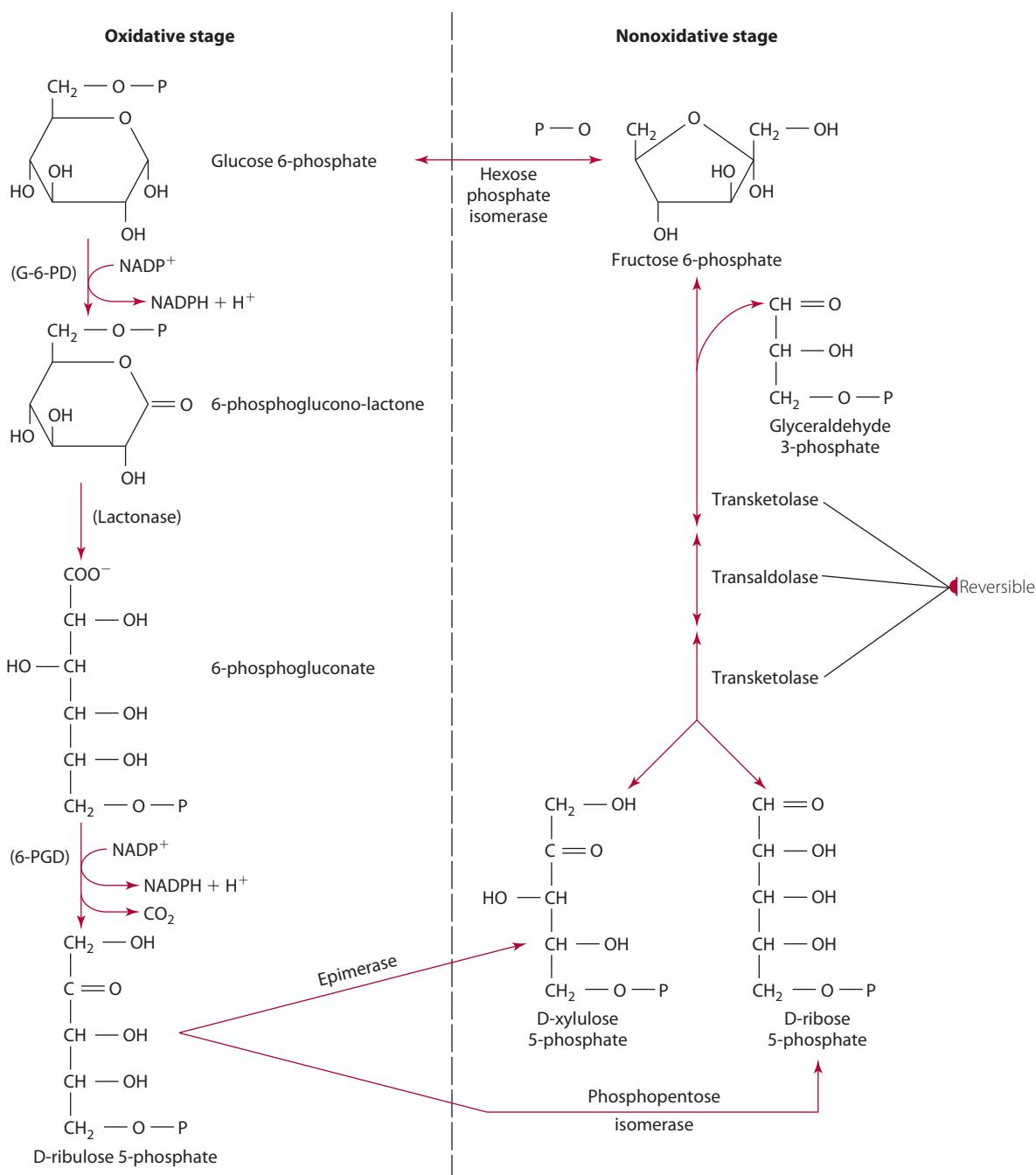


Figure 3.31 The hexosemonophosphate shunt, showing the oxidative stage (left side of diagram) and the nonoxidative stage (right side of diagram). Abbreviations: G-6-PD, glucose 6-phosphate dehydrogenase; 6-PGD, 6-phosphogluconate dehydrogenase.

The reversibility of the transketolase and transaldolase reactions allows hexose phosphates to be converted directly into pentose phosphates, bypassing the oxidative reactions. Therefore, cells that undergo a more rapid rate of replication and that consequently have a greater need for pentose phosphates for nucleic acid synthesis can produce these products in this manner.

The shunt is active in liver, adipose tissue, adrenal cortex, thyroid gland, testes, and lactating mammary gland. Its activity is low in skeletal muscle because of the limited demand for NADPH (fatty acid synthesis) in this tissue and

also because of muscle's reliance on glucose and fatty acids for energy metabolism. Glucose 6-phosphate can be used for either glycolysis or for the pentose phosphate pathway. The choice is made by whether the cell needs energy (ATP/ADP ratio) or for biosynthesis (NADP⁺/NADPH ratio).

GLUCONEOGENESIS

D-glucose is an essential nutrient for most cells to function properly. The brain and other tissues of the central nervous system (CNS) and red blood cells are particularly

dependent on glucose as a nutrient. When dietary intake of carbohydrate is reduced and blood glucose concentration declines, hormones trigger accelerated glucose synthesis from noncarbohydrate sources. Lactate, pyruvate, glycerol (a catabolic product of triacylglycerols), and certain amino acids represent the important noncarbohydrate sources. The process of producing glucose from such compounds is termed gluconeogenesis. The liver is the major site of this activity, although under certain circumstances, such as prolonged starvation, the kidneys become increasingly important in gluconeogenesis.

Gluconeogenesis is essentially a reversal of the glycolytic pathway. Most of the cytoplasmic enzymes involved in the conversion of glucose to pyruvate catalyze their reactions reversibly and therefore provide the means for also converting pyruvate to glucose. Three reactions in the glycolytic sequence are *not* reversible: the reactions catalyzed by the enzymes glucokinase and hexokinase, phosphofructokinase, and pyruvate kinase (sites 1, 3, and 10 in Figure 3.17). These reactions all involve ATP and are unidirectional by virtue of the high, negative free energy change of the reactions. Therefore, the process of gluconeogenesis requires that these reactions be either bypassed or circumvented by other enzyme systems. The presence or absence of these enzymes determines whether a certain organ or tissue is capable of conducting gluconeogenesis. As shown in Figure 3.32, the glucokinase and phosphofructokinase reactions can be bypassed by specific phosphatases (glucose 6-phosphatase and fructose 1,6-bisphosphatase, respectively) that remove phosphate groups by hydrolysis.

The bypass of the pyruvate kinase reaction involves the formation of oxaloacetate as an intermediate. Mitochondrial pyruvate can be converted to oxaloacetate by pyruvate carboxylase, a reaction that was discussed earlier as an anaplerotic process. Oxaloacetate, in turn, can be decarboxylated and phosphorylated to phosphoenolpyruvate (PEP) by PEP carboxykinase, thereby completing the bypass of the pyruvate kinase reaction. The PEP carboxykinase reaction is a cytoplasmic reaction, however, and therefore oxaloacetate must leave the mitochondrion to be acted on by the enzyme. The mitochondrial

membrane, however, is impermeable to oxaloacetate, which therefore must first be converted to either malate (by malate dehydrogenase) or aspartate (by transamination with glutamate; see Chapter 6), both of which freely traverse the mitochondrial membrane. This mechanism is similar to the malate-aspartate shuttle previously discussed. In the cytoplasm, the malate or aspartate can be converted to oxaloacetate by malate dehydrogenase or aspartate aminotransferase (glutamate oxaloacetate transaminase), respectively.

The reactions of the pyruvate kinase bypass also allow the carbon skeletons of various amino acids to enter the gluconeogenic pathway, leading to a net synthesis of glucose. Such amino acids accordingly are called glucogenic. Glucogenic amino acids can be catabolized to pyruvate or to various TCA cycle intermediates or be anaerobically converted to glucose by leaving the mitochondrion in the form of malate or aspartate, as described. Reactions showing the entry of noncarbohydrate substances into the gluconeogenic system are shown in Figure 3.33, along with the bypass of the pyruvate kinase reaction.

Lactate Utilization

Effective gluconeogenesis accounts for the liver's ability to control the high levels of blood lactate that may accompany strenuous physical exertion. Muscle and adipose tissue, for example, lack the ability to form free glucose from noncarbohydrate precursors because they lack glucose 6-phosphatase. Thus, muscle and adipose lactate cannot serve as a precursor for free glucose within these tissues or contribute to the maintenance of blood glucose levels. Also, muscle cells convert lactate to glycogen only very slowly, especially in the presence of glucose (as when glucose enters muscle or adipose cells from the blood). How, then, is the high level of muscle lactate that can be encountered in situations of oxygen debt dealt with? Recovery is accomplished by the gluconeogenic capability of the liver. The lactate leaves the muscle cells and is transported through the general circulation to the liver, where it can be converted to glucose. The glucose can then be returned to the muscle cells to reestablish homeostatic concentrations there. This circulatory transport of

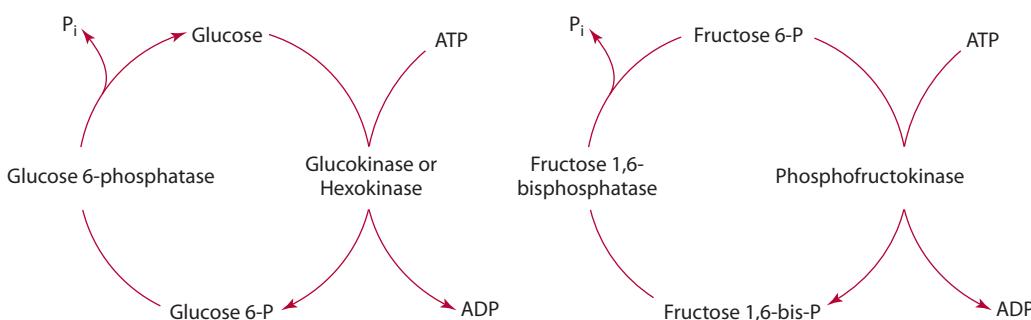


Figure 3.32 Glucokinase and phosphofructokinase reactions.

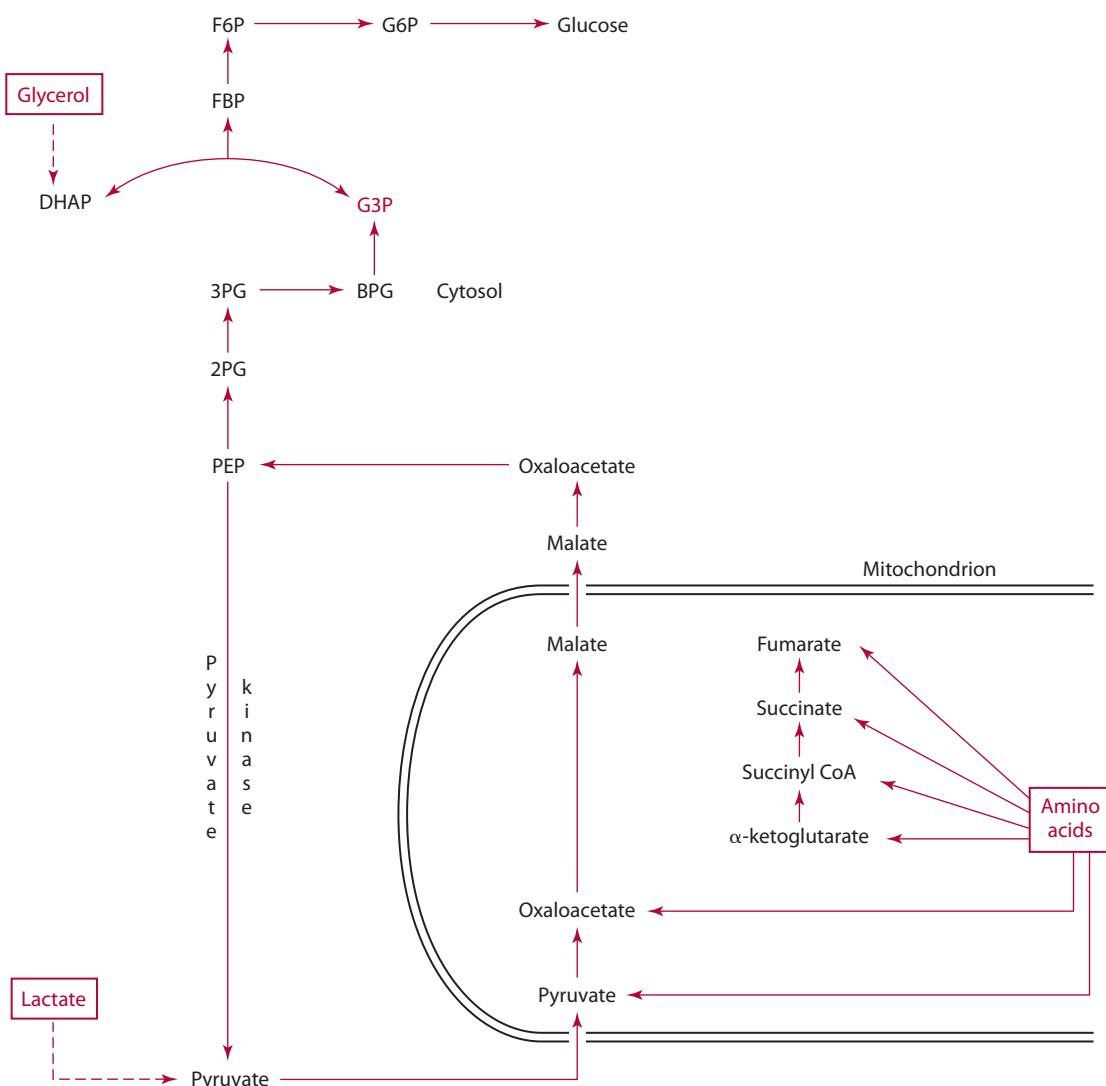


Figure 3.33 The reactions of gluconeogenesis, showing the bypass of the unidirectional pyruvate kinase reaction and the entry into the pathway of noncarbohydrate substances such as glycerol, lactate, and amino acids. Abbreviations: G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde 3-phosphate; BPG, 1,3-bisphosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate.

muscle-derived lactate to the liver and the return of glucose to the muscle is called the Cori cycle.

Efficient Glycogenesis

During the past decade, evidence has emerged from *in vitro* studies that glucose has limited use by the liver as the sole substrate at physiological concentrations and is, in fact, a poor precursor of liver glycogen. However, glucose use is greatly enhanced if additional gluconeogenic substances such as fructose, glycerol, or lactate are available along with the glucose. The term *glucose paradox* refers to the limited incorporation of glucose into glycogen in the absence of other gluconeogenic substances *in vivo*. Glucose ingested during a meal is now believed to take a somewhat round-about path to glycogen. First, it is taken up by red blood cells in the bloodstream and converted to lactate by glycolysis.

Then, the lactate is taken up by the liver and converted to glucose 6-phosphate by gluconeogenesis (and ultimately to glycogen).

Regulation of Metabolism

The purpose of regulation is both to maintain homeostasis and to alter the reactions of metabolism in such a way as to meet the nutritional and biochemical demands of the body. An excellent example is the reciprocal regulation of the glycolysis and TCA cycle (catabolic) pathways and the gluconeogenic (anabolic) pathways. Because the glycolytic conversion of glucose to pyruvate liberates energy, the reversal of the process, gluconeogenesis, must be energy

consuming. The pyruvate kinase bypass in itself is energetically expensive, considering that 1 mol of ATP and 1 mol of GTP must be expended in converting intramitochondrial pyruvate to extramitochondrial PEP. It follows that among the factors that regulate the glycolysis:gluconeogenesis activity ratio is the body's need for energy. Our discussion focuses on the body's energy requirements and on how regulation can speed up or slow down the activity of the metabolic pathways that contribute to release or consumption of energy.

In a broad sense, regulation is achieved by four mechanisms:

- negative or positive modulation of allosteric enzymes by effector compounds
- hormonal activation by covalent modification or induction of specific enzymes
- directional shifts in reversible reactions by changes in reactant or product concentrations
- translocation of enzymes within the cell (covered in Chapter 1)

ALLOSTERIC ENZYME MODULATION

Allosteric enzymes can be stimulated or suppressed by certain compounds, usually formed within the pathway in which the enzymes function. An allosteric, or regulatory, enzyme is said to be positively or negatively modulated by a substance (modulator) according to whether the effect is stimulation or suppression, respectively. Modulators generally act by altering the conformational structure of their allosteric enzymes, causing a shift in the equilibrium between so-called tight and relaxed conformations of the enzyme (these terms refer to the three-dimensional structure of the enzyme). Assume the enzyme is functionally more active in its relaxed form than in its tight form. A positive modulator then causes a shift toward the relaxed configuration, whereas a negative modulator shifts the equilibrium toward the tight form.

As discussed previously in the section on enzymes in Chapter 1, allosteric enzymes catalyze unidirectional, or nonreversible, reactions. The modulators of the enzymes of the undirectional reactions must either stimulate or suppress a reaction in one direction only. Stimulating or suppressing an enzyme that catalyzes both the forward and the reverse direction of a reaction would have little value.

AMP, ADP, and ATP as Allosteric Modulators

Cellular energy has a profound influence on energy-producing cycles. An important regulatory system in energy metabolism is the ratio of the cellular concentration of ADP (or AMP) to ATP. The usual breakdown product of ATP is ADP, but as ADP increases in concentration some of it becomes enzymatically converted to AMP. Therefore, ADP and/or AMP accumulation can signify an excessive breakdown of ATP and a depletion of ATP.

AMP, ADP, and ATP all act as modulators of certain allosteric enzymes, but the effect of AMP or ADP opposes that of ATP. For example, if ATP accumulates, as might occur during a period of muscular relaxation, it negatively modulates certain regulatory enzymes in energy-releasing (ATP-producing) pathways. This effect reduces the production of additional ATP. An increase in AMP (or ADP) concentration conversely signifies a depletion of ATP and the need to produce more of this energy source. In such a case, AMP or ADP can positively modulate allosteric enzymes functioning in energy-releasing pathways as their concentration increases. Two examples of positive modulation by AMP are:

- AMP's ability to bring about a shift from the inactive form of phosphorylase b to an active form of phosphorylase b (Figure 3.16) in glycogenolysis
- AMP's stimulation, by a similar mechanism, of the enzyme phosphofructokinase, which catalyzes a reaction in the glycolytic pathway

It can be reasoned that increased levels of AMP are accompanied by an enhanced activity of either of these reactions that encourages glucose catabolism. The resulting shift in metabolic direction, as signaled by the AMP buildup, causes the release of energy as glucose is metabolized and helps restore depleted ATP stores.

In addition to being positively modulated by AMP, phosphofructokinase is modulated positively by ADP and negatively by ATP. As the store of ATP increases and further energy release is not called for, ATP can thus signal the slowing of the glycolytic pathway at that reaction. Phosphofructokinase is an extremely important rate-controlling allosteric enzyme and is modulated by a variety of substances. Its regulatory function has already been described in Chapter 1.

Other regulatory enzymes in carbohydrate metabolism are modulated by ATP, ADP, or AMP. Pyruvate dehydrogenase complex, citrate synthase, and isocitrate dehydrogenase are all negatively modulated by ATP. Pyruvate dehydrogenase complex is positively modulated by AMP, and citrate synthase and isocitrate dehydrogenase are positively modulated by ADP.

REGULATORY EFFECT OF NADH:NAD⁺ RATIO

The ratio of NADH to NAD⁺ also has an important regulatory effect. Certain allosteric enzymes are responsive to an increased level of NADH or NAD⁺. These coenzymes regulate their own formation through negative modulation. Because NADH is a product of the oxidative catabolism of carbohydrate, its accumulation would signal for a decrease in catabolic pathway activity. Conversely, higher proportions of NAD⁺ signify that a system is in an elevated state of oxidation readiness and would send a modulating signal to accelerate catabolism. Stated differently, the level of NADH in the

fasting state is markedly lower than in the fed state because the rate of its reoxidation by electron transport would exceed its formation from substrate oxidation. Fasting, therefore, logically encourages glycolysis and TCA cycle oxidation of carbohydrates. Dehydrogenase reactions, which involve the interconversion of the reduced and oxidized forms of the cosubstrate, are reversible. If metabolic conditions cause either NADH or NAD⁺ to accumulate, the equilibrium is shifted so as to consume more of the predominant form. Pyruvate dehydrogenase complex is positively modulated by NAD⁺, whereas pyruvate kinase, citrate synthase, and α -ketoglutarate dehydrogenase are negatively modulated by NADH.

HORMONAL REGULATION

Hormones can regulate specific enzymes either by covalent regulation or by enzyme induction. Covalent regulation refers to the binding of a group by a covalent bond. An example of this type of regulation is the phosphorylation and dephosphorylation of the enzymes, which converts them to active or inactive forms. In some instances, phosphorylation activates and dephosphorylation inactivates the enzyme. In other cases, the reverse may be true. Examples may be found in the covalent regulation of glycogen synthase and glycogen phosphorylase, enzymes discussed in the sections on glycogenesis and glycogenolysis, respectively. Phosphorylation inactivates glycogen synthase, whereas dephosphorylation activates it. In contrast, phosphorylation activates glycogen phosphorylase, and dephosphorylation inactivates it.

Another important example of covalent regulation by a hormone is the control by glucagon of the relative rates of liver glycolysis and gluconeogenesis. The control is directed at the opposing reactions of the phosphofructokinase (PFK) and fructose bisphosphatase (FBPase) site and is mediated through a compound called fructose 2,6-bisphosphate. Unlike fructose 1,6-bisphosphate, fructose 2,6-bisphosphate is not a normal glycolysis intermediate but instead serves solely as a regulator of pathway activity. *Fructose 2,6-bisphosphate stimulates PFK activity and suppresses FBPase activity, thereby stimulating glycolysis and reducing gluconeogenesis.* Cellular concentration of fructose 2,6-bisphosphate is set by the relative rates of its formation and breakdown. The compound is formed by phosphorylation of fructose 6-phosphate by phosphofructokinase 2 (PFK-2) and is broken down by fructose bisphosphatase 2 (FBPase-2). The designation 2 distinguishes these enzymes from PFK and FBPase, which catalyze the formation and breakdown, respectively, of fructose 1,6-bisphosphate. PFK-2 and FBPase-2 activities are expressed by a single (bifunctional) enzyme, and the relative activity of each is controlled by glucagon. Glucagon stimulates the phosphorylation of the bifunctional enzyme, resulting in sharply increased FBPase-2 activity and suppression of PFK-2 activity. *Glucagon therefore stimulates hepatic gluconeogenesis and suppresses glycolysis by reducing the concentration of fructose*

2,6-bisphosphate, a positive modulator of the glycolytic enzyme PFK. The end result is that in response to falling blood glucose levels, the release of glucagon encourages hepatic gluconeogenesis and thus helps to restore blood glucose levels.

Covalent regulation is usually mediated through cAMP, which acts as a second messenger in the hormones' action on the cell. Recall that insulin strongly affects the glycogen synthase reaction positively and that epinephrine and glucagon positively regulate glycogen phosphorylase in muscle and the liver, respectively. Each of these hormonal effects is mediated through covalent regulation.

The control of enzyme activity by hormone induction represents another mechanism of regulation. Enzymes functioning in the glycolytic and gluconeogenic pathways can be divided into three groups:

Group 1: Glycolytic enzymes

- Glucokinase
- Phosphofructokinase
- Pyruvate kinase

Group 2: Bifunctional enzymes

- Phosphoglucoisomerase
- Aldolase
- Triosephosphate isomerase
- Glyceraldehyde 3-phosphate dehydrogenase
- Phosphoglycerate kinase
- Phosphoglyceromutase
- Enolase
- Lactate dehydrogenase

Group 3: Gluconeogenic enzymes

- Glucose 6-phosphatase
- Fructose bisphosphatase
- PEP carboxykinase
- Pyruvate carboxylase

As discussed in Chapter 1, enzyme (protein) synthesis is either constitutive (at a constant rate) or adaptive (in response to a stimulus, i.e., inductive). Groups 1 and 3 are inducible enzymes, meaning that their concentrations can rise and fall in response to molecular signals such as a sustained change in the concentration of a certain metabolite. Such a change might arise through a prolonged shift in the dietary intake of certain nutrients. Induction stimulates transcription of new messenger RNA, programmed to produce the hormone. Glucocorticoid hormones are known to stimulate gluconeogenesis by inducing the key gluconeogenic enzymes to form, and insulin may stimulate glycolysis by inducing increased synthesis of key glycolytic enzymes. Enzymes in group 2 are not inducible and are produced at a steady rate under the control of constitutive, or basal, gene systems. Noninducible enzymes are required all the time at a relatively constant level of activity, and their genes are expressed at a more or less constant level in virtually all cells. Genes for enzymes that are not inducible are sometimes called housekeeping genes.

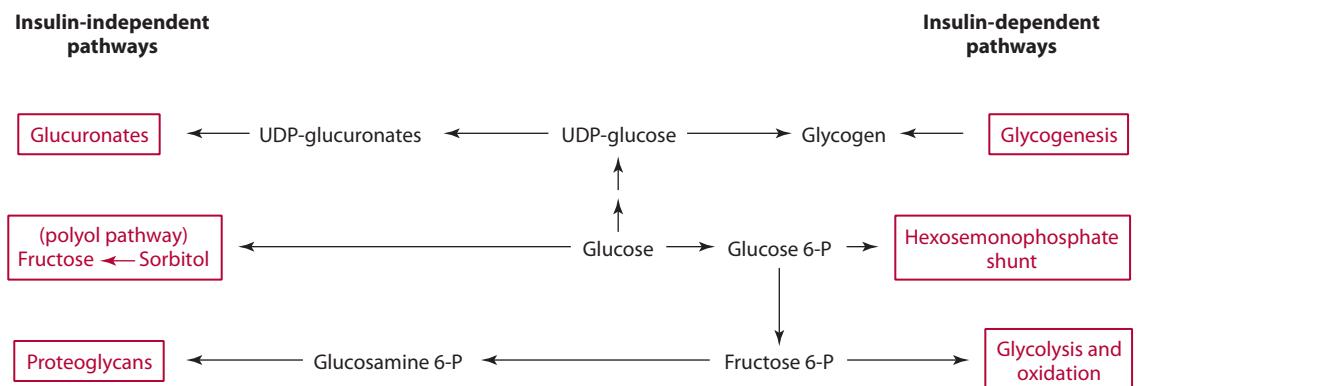


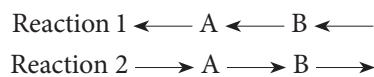
Figure 3.34 Insulin-independent and insulin-dependent pathways of glucose metabolism.

The interrelationship among pathways of carbohydrate metabolism is exemplified by the regulation of blood glucose concentration. The interconversion of the pathways, a topic of Chapter 7, is best understood after metabolism of lipids and amino acids has been discussed (Chapters 5 and 6). Largely through the opposing effects of insulin and glucagon, fasting serum glucose level normally is maintained within the approximate range of 60 to 90 mg/dL (3.3–5.0 μmol/L). Whenever blood glucose levels are excessive or sustained at high levels because insulin is insufficient, other insulin-independent pathways of carbohydrate metabolism for lowering blood glucose become increasingly active. Such insulin-independent pathways are indicated in Figure 3.34. The overactivity of these pathways in certain tissues is believed to be partly responsible for the clinical manifestations of diabetes mellitus, type 1 (see the Perspectives in Chapters 7 and 8).

DIRECTIONAL SHIFTS IN REVERSIBLE REACTIONS

Most enzymes catalyze reactions reversibly, and the preferred direction a reversible reaction is undergoing at a particular moment is largely dependent on the relative concentration of each reactant and product. An increasing concentration of one of the reactants drives or forces the

reaction toward forming the other. For example, consider the hypothetical pathway intermediates A and B, which are interconverted reversibly.



Reaction 1 may represent the reaction in a metabolic steady state in which the formation of A from B is preferred over the formation of B from A. It shows a net formation of A from B, as indicated by the size of the directional arrows. Reaction 2 shows that the steady state shifts toward the formation of B from A if some metabolic event or demand causes the concentration of A to rise above its homeostatic levels.

This concept is exemplified by the phosphoglucomutase reaction, which interconverts glucose 6-phosphate and glucose 1-phosphate and which functions in the pathways of glycogenesis and glycogenolysis (Figures 3.13 and 3.15). At times of heightened glycogenolytic activity (rapid breakdown of glycogen), glucose 1-phosphate concentration rises sharply, driving the reaction toward the formation of glucose 6-phosphate. With the body at rest, glycogenesis and gluconeogenesis are accelerated, increasing the concentration of glucose 6-phosphate. This increase in turn shifts the phosphoglucomutase reaction toward the formation of glucose 1-phosphate and ultimately glycogen.

SUMMARY

This chapter has dealt with a subject of vital importance in nutrition: the conversion of the energy contained within nutrient molecules into energy usable by the body. It examines an important food source of that energy, carbohydrates. The major sources of dietary carbohydrate are the starches and the disaccharides. In the course of digestion, these are hydrolyzed by specific glycosidases to their component monosaccharides, which are absorbed into the circulation from the intestine. The monosaccharides then

are transported to the cells of various tissues, passing through the cells' outer membrane by facilitative transport by way of transporters. Glucose is transported into the cells of many different tissues by the GLUT family of transporters. In the cells, monosaccharides first are phosphorylated at the expense of ATP and then can follow any of several integrated pathways of metabolism. Glucose is phosphorylated in most cells by hexokinase, but in the liver it is phosphorylated by glucokinase. Fructose

is phosphorylated mainly by fructokinase in the liver. Galactose is phosphorylated by galactokinase, also a liver enzyme.

Cellular glucose can be converted to glycogen, primarily in liver and skeletal muscle, or it can be routed through the energy-releasing pathways of glycolysis and the tricarboxylic acid cycle (TCA cycle) in these and other tissues for ATP production. Glycolytic reactions convert glucose (or glucose residues from glycogen) to pyruvate. From pyruvate, either an aerobic course (complete oxidation in the TCA cycle) or an anaerobic course (to lactate) can be followed. Nearly all the energy formed by the oxidation of carbohydrates to CO_2 and H_2O is released in the TCA cycle, as reduced coenzymes are oxidized by mitochondrial electron transport. On complete oxidation, approximately 40% of this energy is retained in the high-energy phosphate bonds of ATP. The remaining energy supplies heat to the body.

Noncarbohydrate substances derived from the other major nutrients, the glycerol from triacylglycerols (fats) and certain amino acids, can be converted to glucose or glycogen by the pathways of gluconeogenesis. The basic carbon skeleton of fatty acids cannot be converted to a net synthesis of glucose, but some of the carbons from fatty acids find their way into the carbohydrate molecule. In gluconeogenesis, the reactions are basically the reversible reactions of glycolysis, shifted toward glucose synthesis in accordance with reduced energy demand by the body. Three kinase reactions occurring in glycolysis are not reversible, however, requiring the involvement of different enzymes and pathways to circumvent those reactions in the process of gluconeogenesis. Muscle glycogen provides a source of glucose for energy only for muscle fiber in which it is stored, because muscle lacks the enzyme glucose 6-phosphatase, which forms free glucose from glucose 6-phosphate. Glucose 6-phosphatase is active in the liver, however, which means that the liver can release free glucose from its glycogen stores into the circulation for maintaining blood glucose and for use by other tissues. The Cori cycle describes the liver's uptake and gluconeogenic conversion of muscle-produced lactate to glucose.

A metabolic pathway is regulated according to the body's need for energy or for maintaining homeostatic cellular concentrations of certain metabolites. Regulation is exerted mainly through hormones, through substrate concentrations (which can affect the velocity of enzyme reactions), and through allosteric enzymes that can be modulated negatively or positively by certain pathway products.

In Chapters 5 and 6, we will see that fatty acids and the carbon skeleton of various amino acids also are ultimately oxidized through the TCA cycle. The amino acids that do become TCA cycle intermediates, however, may not be completely oxidized to CO_2 and H_2O but instead may leave the cycle to be converted to glucose or glycogen (by

gluconeogenesis) should dietary intake of carbohydrate be low. The glycerol portion of triacylglycerols enters the glycolytic pathway at the level of dihydroxyacetone phosphate, from which point it can be oxidized for energy or used to synthesize glucose or glycogen. The fatty acids of triacylglycerols enter the TCA cycle as acetyl CoA, which is oxidized to CO_2 and H_2O but cannot contribute carbon for the net synthesis of glucose. This topic is considered further in Chapter 5.

These examples of the entrance of noncarbohydrate substances into the pathways discussed in this chapter are cited here to remind the reader that these pathways are not singularly committed to carbohydrate metabolism. Rather, they must be thought of as common ground for the interconversion and oxidation of fats and proteins as well as carbohydrate. Maintaining this broad perspective will be essential when we move on to Chapters 5 and 6, which examine the metabolism of lipids and proteins, respectively.

Much of the energy needs for the body is in the form of stored ATP. ATP can be generated by two distinct mechanisms:

1. The transfer of a phosphate group from a very high energy phosphate donor to ADP, a process called substrate-level phosphorylation.
2. Oxidative phosphorylation, by which the energy derived from the translocation of H^+ , which occurs during mitochondrial electron transport, is used to phosphorylate ADP to form ATP.

Oxidative phosphorylation is the major route for ATP production. Electron flow in the electron transport chain is from reduced cosubstrates to molecular oxygen. Molecular oxygen becomes the ultimate oxidizing agent and becomes H_2O in the process. The down-hill flow of electrons and proton translocation generate sufficient energy to affect oxidative phosphorylation at multiple sites along the chain. The energy from this process that is not conserved as chemical energy (ATP) is given off as heat. About 60% of the energy is in the form of heat.

Carbohydrate metabolism, including the energy-releasing, systematic oxidation of glucose to CO_2 and H_2O , exemplify reactions of substrate-level and oxidative phosphorylation. Similar energy transfer happens with the lipid and amino acid pathways whenever a dehydration reaction occurs.

The hexosemonophosphate shunt generates important intermediates not produced in other pathways of the body, such as pentose phosphates for RNA and DNA synthesis and the production of NADPH, which is used in the synthesis of fatty acids and in drug metabolism.

This chapter begins the important topic in nutrition of the regulation of metabolism. The primary mechanisms discussed are by allosteric modulation of enzyme

activity (either negative or positive), hormonal activation by covalent modification, directional shifts in reversible reactions, and the translocation of enzymes. This topic will be revisited several times in Chapters 7 and 8.

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Understanding the integration of metabolism and the control of energy balance is important. Much of the effects of exercise, disease, weight loss, and weight gain can be explained with these principles.

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Suggested Readings

McGarry JD, Kuwajima M, Newgard CB, Foster DW. From dietary glucose to liver glycogen: The full circle round. *Ann Rev Nutr* 1987; 7:51–73.

The glucose paradox is emphasized, from the standpoint of its emergence, as are the attempts to resolve it.

Pilkis SJ, El-Maghrabi MR, Claus TH. Hormonal regulation of hepatic gluconeogenesis and glycolysis. *Ann Rev Biochem* 1988; 57:755–83.

This is a brief, clearly presented summary of the effect of certain hormones on the important regulatory enzymes in these major pathways of carbohydrate metabolism.

Web Sites

www.ncbi.nlm.gov/books

The NCBI Bookshelf is a growing collection of biomedical books that can be searched directly by typing a concept into the textbox in the web site.

www.nlm.nih.gov/books

National Library of Medicine

www.medscape.com/home

From WebMD. Provides specialty information and education for physicians and other health professionals.

www.cdc.gov

Centers for Disease Control and Prevention

www.ama-assn.org

American Medical Association

www.wadsworth.com/nutrition

Wadsworth Publishing Company

www.hopkinsmedicine.org

A Web site from John Hopkins School of Medicine

Hypoglycemia: Fact or Fall Guy?

Maintaining normal blood glucose concentration (normoglycemia) is essential to good health. The consequences of abnormally high levels (**hyperglycemia**), such as occur in diabetes, are well established. Concentrations that are significantly below the normal range (**hypoglycemia**) introduce a well-recognized syndrome as well. This Perspective deals with the consequences of hypoglycemia.

Glucose is our most important carbohydrate nutrient. Its concentration in the blood is established by a balance between processes that infuse glucose into the blood and those that remove it from the blood for use by the cells. The primary sources of blood glucose are

- exogenous (dietary sugars and starches)
- hepatic glycogenolysis
- hepatic gluconeogenesis

The primary glucose-requiring tissues are the brain, erythrocytes, and muscle.

Preprandial (before-meals) serum glucose levels generally range from 70 to 105 mg/dL in patients who have no disorder of glucose metabolism (1 dL = 100 mL). If whole blood is the specimen, the range is somewhat lower, approximately 60 to 90 mg/dL. Should levels drop below this range, glucoreceptors in the hypothalamus stimulate the secretion of counter regulatory hormones to try to return the glucose to homeostatic levels. Such hormones include

- glucagon, which increases hepatic glycogenolysis and gluconeogenesis
- epinephrine, which inhibits glucose use by muscle and increases muscle glycogenolysis
- cortisol and growth hormone, although these have a delayed release and do not contribute significantly to acute recovery

The major hormone acting antagonistically to those listed is insulin, which has the opposing effect of increasing cellular uptake of glucose by muscle and adipose tissue, thereby reducing its serum concentration.

If, in spite of counterregulatory hormone response, subnormal concentrations of glucose persist, a state of clinical hypoglycemia can possibly result. This state, in turn, can give rise to any number of associated symptoms. Symptoms may be attributable to the low glucose level or to the hormonal response to the low glucose level and are broadly categorized as adrenergic or neuroglucopenic.

Adrenergic symptoms arise as a result of increased activity of the autonomic nervous system, coincident with accelerated release of epinephrine. Adrenergic symptoms include weakness, sweating/warmth, tachycardia (rapid heart rate), palpitation, and tremor.

Neuroglucopenic symptoms are usually associated with a more severe hypoglycemic state. They include headache, hypothermia, visual disturbances, mental dullness, and seizures. During insulin-induced hypoglycemia, adrenergic symptoms may manifest at serum glucose concentrations of about 60 mg/dL, and neuroglucopenic symptoms at approximately 45 to 50 mg/dL.

Many of the symptoms listed here are nonspecific and somewhat vague, and they may arise as a result of any number of unrelated disorders. For this reason, hypoglycemia is among the most overdiagnosed ailments. Before it can be diagnosed as true clinical hypoglycemia, a condition must satisfy these criteria:

- low serum glucose level
- presence of adrenergic or neuroglucopenic symptoms
- relief of symptoms upon ingesting carbohydrate and a return of glucose levels toward normal

Patients commonly may have serum glucose concentrations as low as 50 mg/dL and yet be asymptomatic (without symptoms), whereas others may be normoglycemic but have symptoms congruent with hypoglycemia. In neither case is true clinical hypoglycemia verifiable. Two types of hypoglycemia exist:

- *Fasting hypoglycemia* is usually caused by drugs, such as exogenous insulin, used to treat type 1 diabetes, or the sulfonylureas, which stimulate

insulin secretion. It can also be caused by insulinomas (β cell tumors) or excessive intake of alcohol.

■ *Fed (reactive) hypoglycemia* has two possible causes in patients who have not undergone certain gastrointestinal surgical procedures: impaired glucose tolerance (IGT) and the more common idiopathic postprandial syndrome.

Some patients with IGT or early stages of diabetes experience **postprandial** (after-meals) hypoglycemia because insulin response to food is initially delayed and is followed by excessive insulin release that drives glucose levels down to hypoglycemic concentrations. This condition can be diagnosed by the oral glucose tolerance test (OGTT), in which serum glucose levels are observed following an oral load of glucose over an extended period of time (often as long as 5 hours).

Much attention has been paid to the putative idiopathic (of unknown origin) postprandial form of hypoglycemia. It has been difficult to document that adrenergic symptoms occur simultaneously with the analytical finding of hypoglycemia. As discussed earlier, adrenergic symptoms frequently do not correlate with low glucose levels. Furthermore, feeding may not relieve the symptoms or elevate serum glucose. This is the form of hypoglycemia that has been over diagnosed as such. The actual cause is more complex, and other factors may be players in the game.

Diet therapy is the cornerstone of treatment for all forms of reactive (fed) hypoglycemia. Patients should avoid simple or refined carbohydrates and may also benefit from frequent, small feedings of snacks containing a mixture of carbohydrate, protein, and fat.

Suggested Reading

Andreoli T, Bennett J, Carpenter C, and Plum F. Hypoglycemia. In: Cecil Essentials of Medicine, 5th ed. Philadelphia: W. B. Saunders, 2001, Chap. 69.

Web Site

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4

Fiber

Definitions of Dietary Fiber and Functional Fiber

Fiber and Plants

Chemistry and Characteristics of Dietary and Functional Fibers

Cellulose

Hemicellulose

Pectins

Lignin

Gums

β-glucans

Fructans—Inulin, Oligofructose, and Fructooligosaccharides

Resistant Starch

Chitin and Chitosan

Polydextrose and Polyols

Psyllium

Resistant Dextrans

Selected Properties and Physiological and Metabolic Effects of Fiber

Solubility in Water

Water-Holding/Hydration Capacity and Viscosity

Adsorption or Binding Ability

Degradability/Fermentability

Roles of Fiber in Disease Prevention and Management

Recommended Fiber Intake

PERSPECTIVE

Phytochemicals and Herbal Supplements in Health and Disease

Dietary fiber was recognized again as an important food component in about the mid-1970s. Yet the concept of fiber, originally called crude fiber or indigestible material, and its extraction from animal feed and forages were introduced in Germany during the 1850s. The crude fiber extraction method was used, even for human food, into the 1990s, despite the existence of better methodology and the inconsistent relationship between crude fiber and dietary fiber. Today, soluble and insoluble fibers may be extracted or, in some cases, manufactured in laboratories and added as an ingredient to create foods that contain what we now call *functional fiber*.

Results from extensive research devoted to dietary fiber during the last 25 or so years have found that fiber is important for gastrointestinal tract function and for preventing and managing a variety of diseases. The varied effects of fiber observed by researchers are related to the fact that dietary fiber consists of different components, each with its own distinctive characteristics. Examining these many components and their various distinctive characteristics emphasizes the fact that dietary fiber cannot be considered a single entity. This chapter addresses the definitions of dietary fiber and functional fiber; the relationship between plants and fiber; and the chemistry, intraplant functions, and properties of fiber. It also reviews recommendations for fiber intake.

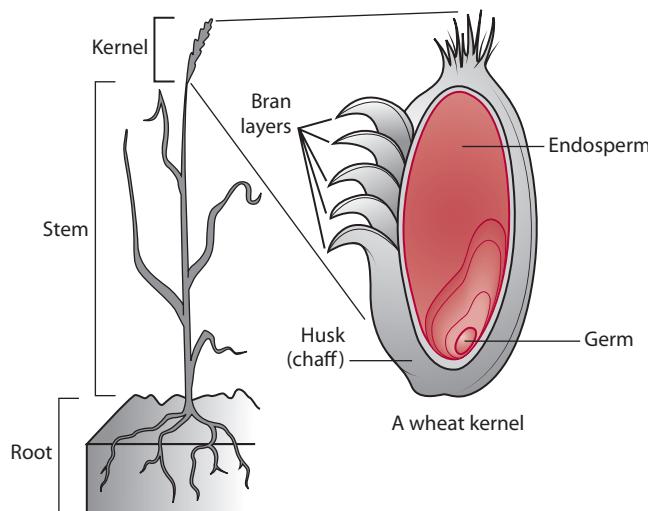
Definitions of Dietary Fiber and Functional Fiber

With the publication of the 2002 Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Protein, and Amino Acids by the National Academy of Sciences Food and Nutrition Board, uniform definitions for dietary fiber and functional fiber were established. **Dietary fiber** refers to nondigestible (by human digestive enzymes) carbohydrates and lignin that are intact and intrinsic in plants [1]. **Functional fiber** consists of nondigestible carbohydrates that have been isolated, extracted, or manufactured and have been shown to have beneficial physiological effects in humans [1]. Table 4.1 lists dietary and functional fibers. Each fiber listed in Table 4.1 is discussed in this chapter in the section “Chemistry and Characteristics of Dietary and Functional Fibers.”

Table 4.1 Dietary and Functional Fibers

Dietary Fibers	Functional Fibers
Cellulose	Cellulose
Hemicellulose	Pectin
Pectin	Lignin*
Lignin	Gums
Gums	β -glucans
β -glucans	Fructans*
Fructans	Chitin and chitosan*
Resistant starches	Polydextrose and polyols*
	Psyllium
	Resistant dextrins*
	Resistant starches

*Data showing positive physiological effects in humans are needed.

**Figure 4.1** The partial anatomy of a wheat plant.

The plant cell wall consists of both a primary and a secondary wall and contains >95% of dietary fibers. The primary wall is a thin envelope that surrounds the contents of the growing cell. The secondary wall develops as the cell matures. The secondary wall of a mature plant contains many strands of cellulose arranged in an orderly fashion within a matrix of noncellulosic polysaccharides. The primary wall also contains cellulose, but in smaller amounts and less well organized. The hemicellulose content of plants varies but can make up 20% to 30% of the cell walls. Starch, the energy storage product of the cell, is found within the cell walls. Lignin deposits form in specialized cells whose function is to provide structural support to the plant. As the plant matures, lignin spreads through the intracellular spaces, penetrating the pectins. Pectins function as intercellular cement and are located between and around the cell walls. Lignin continues dispersing through intracellular spaces, but it also permeates the primary wall and then spreads into the developing secondary wall. As plant development continues further, suberin is deposited in the cell wall just below the epidermis and skin. Suberin is made up of a variety of substances, including phenolic compounds as well as long-chain alcohols and polymeric esters of fatty acids. Cutin, also made of polymeric esters of fatty acids, is a water-impermeable substance that is secreted onto the plant surface. Both suberin and cutin are enzyme- and acid-resistant. In addition to these substances, waxes (which consist of complex hydrophobic, hydrocarbon compounds) are found in many plants, coating the external surfaces.

Consuming plant foods provides fiber in the diet. The plant species, the part of the plant (leaf, root, stem), and the plant's maturity all influence the composition (cellulose, hemicellulose, pectin, lignin, etc.) of the fiber that is consumed. Figure 4.1 shows the anatomy of a wheat plant. Consuming cereal such as wheat bran (which consists of

the outer layers of cereal grains as shown in Figure 4.1) provides primarily hemicellulose along with lignin. Eating fruits and vegetables provides almost equal quantities (~30%) of cellulose and pectin. In contrast, cereals are quite low in cellulose. This chapter reviews each of the dietary fibers, including their characteristics and functions, as well as foods rich in the particular fiber. Identifying the chemical characteristics and various intraplant functions and properties of these plant cell wall substances (or substances in contact with the wall) helps us to conceptualize how fiber components may affect physiological and metabolic functions in humans.

Chemistry and Characteristics of Dietary and Functional Fibers

CELLULOSE

Cellulose is considered a dietary fiber as well as a functional fiber when added to foods. Chemical analysis shows cellulose (Figure 4.2a) to be a long, linear **polymer** (a high-molecular-weight substance made up of a chain of repeating units) of β 1-4 linked glucose units (alternately stated, a D-glucopyranosyl homopolymer in β 1-4 glycosidic linkages). Cellulose is a main component of plant cell walls. Hydrogen bonding between sugar residues in adjacent parallel running cellulose chains imparts a microfibril three-dimensional structure to cellulose. Being a large, linear, neutrally charged molecule, cellulose is water insoluble, although it can be modified chemically (e.g., carboxymethylcellulose, methylcellulose, and hydroxypropylmethylcellulose) to be more water soluble for use as a food additive. The extent to which cellulose is degraded

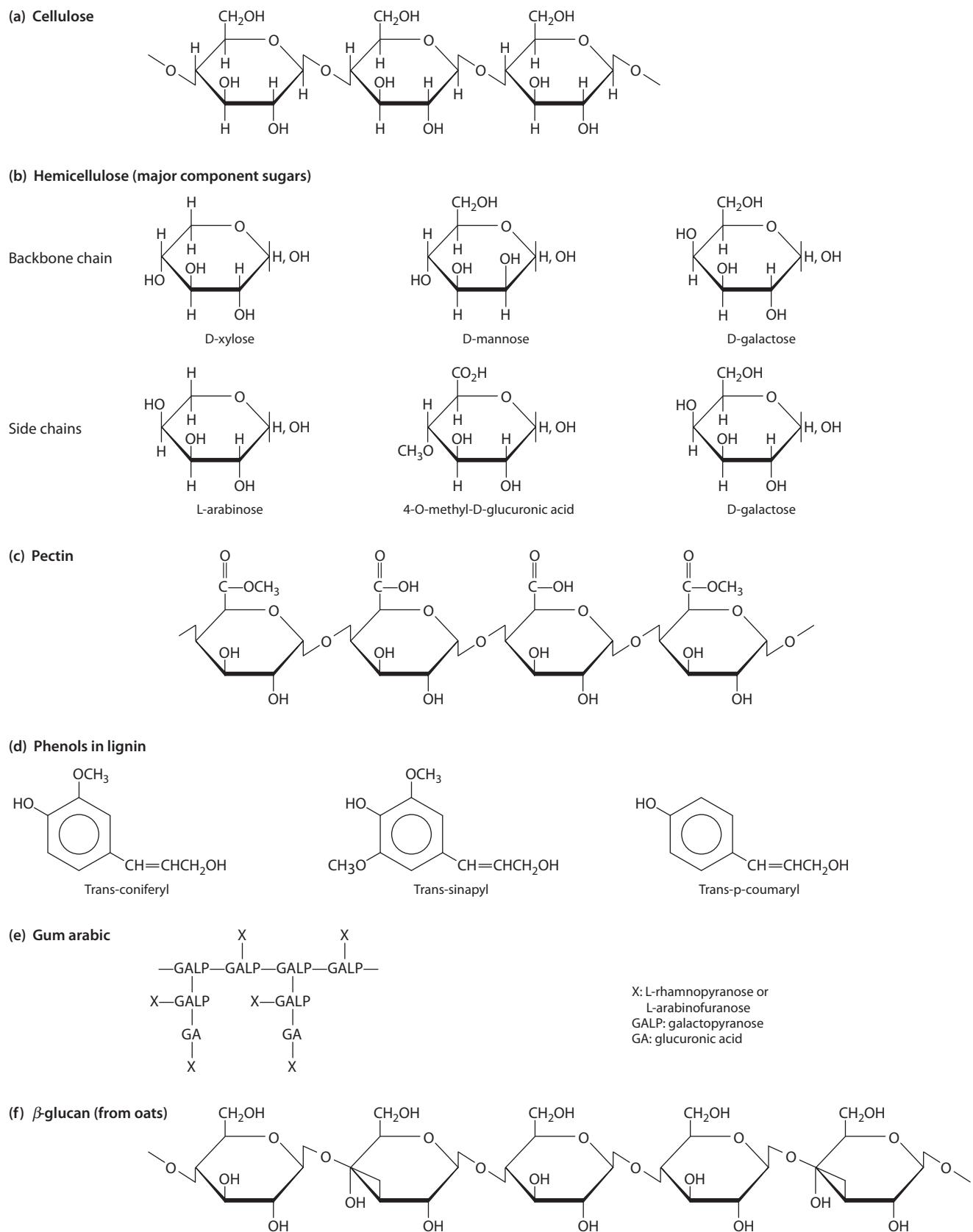


Figure 4.2 Chemical structures of dietary fibers and some functional fibers.

by colonic bacteria varies, but generally it is poorly fermented. Some examples of foods high in cellulose relative to other fibers include bran, legumes, nuts, peas, root vegetables, vegetables of the cabbage family, the outer covering of seeds, and apples. Purified, powdered cellulose (usually isolated from wood) and modified cellulose are often added to foods, for example, as a thickening or texturing agent or to prevent caking or syneresis (leakage of liquid). Some examples of foods to which cellulose or a modified form of cellulose is added include breads, cake mixes, sauces, sandwich spreads, dips, frozen meat products (e.g., chicken nuggets), and fruit juice mixes.

HEMICELLULOSE

Hemicellulose, a dietary fiber and a component of plant cell walls, consists of a heterogeneous group of polysaccharide substances that vary both between different plants and within a plant depending on location. Hemicelluloses contain a number of sugars in their backbone and side chains. The sugars, which form a basis for hemicellulose classification, include xylose, mannose, and galactose in the hemicellulose backbone and arabinose, glucuronic acid, and galactose in the hemicellulose side chains. The number of sugars in the side chains varies such that some hemicelluloses are relatively linear, whereas others are highly branched. Some of the sugars found in hemicelluloses are shown in Figure 4.2b. One example of a hemicellulose structure is β 1-4 linked D-xylopyranose units with branches of 4-O-methyl D-glucopyranose uronic acids linked by α 1-2 bonds or with branches of L-arabinofuranosyl units linked by α 1-3 bonds. The sugars in the side chains confer important characteristics on the hemicellulose. For example, hemicelluloses that contain acids in their side chains are slightly charged and water soluble. Other hemicelluloses are water insoluble. Fermentability of the hemicelluloses by intestinal microflora (bacteria adapted to living in that specific environment) is also influenced by the sugars and their positions. For example, hexose and uronic acid components of hemicellulose are more accessible to bacterial enzymes than are the other hemicellulose sugars. Foods that are relatively high in hemicellulose include bran and whole grains as well as nuts, legumes, and some vegetables and fruits.

PECTINS

Pectins are a family of compounds that in turn make up a larger family of pectic substances including pectins, pectic acids, and pectinic acids. Pectin is both a dietary fiber and a functional fiber. Pectinic acids represent polygalacturonic acids that either are partly esterified with methanol or have no or negligible amounts of methyl esters. Pectins typically represent a complex group of polysaccharides called galacturonoglycans, which also vary in methylester content.

Galacturonic acid is a primary constituent of pectin and makes up its backbone structure. Pectin's backbone is usually an unbranched chain of α 1-4-linked D-galacturonic acid units (polygalactopyranosyluronic acids), as shown in Figure 4.2c. Many of the carboxyl groups of the uronic acid moieties exist as methyl esters. Other carbohydrates may be linked to the galacturonic acid chain. These additional sugars, sometimes found attached as side chains, include rhamnose, arabinose, xylose, fucose, and galactose; galactose may be present in a methylated form. Pectins form part of the primary cell wall of plants and part of the middle lamella. They are water soluble and gel-forming and have high ion-binding potential. Because they are stable at low pH values, pectins perform well in acidic foods. In the body, pectins are almost completely metabolized by colonic bacteria and are not a good fecal bulking agent. Rich sources of pectins include apples, strawberries, and citrus fruits. Legumes, nuts, and some vegetables also provide pectins. Commercially, pectins are usually extracted from citrus peel or apples and added to many products. Pectin is added to jellies and jams to promote gelling. It is also added to fruit roll-ups, fruit juices, and icing or frosting, among other products. Pectin is added to some enteral nutrition formulas administered to tube-fed hospital patients to provide a source of fiber in their diets.

LIGNIN

Lignin is a three-dimensional (highly branched) polymer composed of phenol units with strong intramolecular bonding. The primary phenols that compose lignin include trans-coniferyl, trans-sinapyl, and trans-p-coumaryl, shown in Figure 4.2d. Lignin forms the structural components of plants and is thought to attach to other noncellulose polysaccharides such as heteroxylans found in plant cell walls. Lignin is insoluble in water, has hydrophobic binding capacity, and is generally poorly fermented by colonic bacterial microflora. Some studies, however, report metabolism by gut flora to form the lignan enterolactone, a weak phytoestrogen [2]. Lignin is both a dietary fiber and a functional fiber. It is found especially in the stems and seeds of fruits and vegetables and in the bran layer of cereals. More specific examples of foods high in lignin include wheat, mature root vegetables such as carrots, and fruits with edible seeds such as strawberries.

GUMS

Gums, also called *hydrocolloids*, represent a group of substances. Gums are secreted at the site of plant injury by specialized secretory cells and can be exuded from plants (i.e., forced out of plant tissues). Gums that originate as tree **exudates** include gum arabic, gum karaya, and gum ghatti; gum tragacanth is a shrub exudate. Gums are composed of a variety of sugars and sugar derivatives. Galactose and

glucuronic acid are prominent, as are uronic acids, arabinose, rhamnose, and mannose, among others. Within the large intestine, gums are highly fermented by colonic bacteria. Of the tree exudates, gum arabic is most commonly used as a food additive to promote gelling, thickening, and stabilizing. Gum arabic, shown in Figure 4.2e, contains a main galactose backbone joined by β 1-3 linkages and β 1-6 linkages along with side chains of galactose, arabinose, rhamnose, glucuronic acid, or methylglucuronic acid joined by β 1-6 linkages. The nonreducing ends terminate with a rhamnopyrosyl unit. The popularity of gum arabic is attributable to its physical properties, which include high water solubility, pH stability, and gelling characteristics. It is found in candies such as caramels, gumdrops, and toffees, as well as in other assorted products.

The water-soluble gums guar gum and locust bean gum (also called carob gum) are made from the ground endosperm of guar seeds and locust bean seeds, respectively. These gums consist mostly of galactomannans, the main component of the endosperm. Galactomannans contain a mannose backbone in 1-4 linkages and in a 2:1 or 4:1 ratio with galactose present in the side chains. Guar galactomannans have more branches than locust bean galactomannans.

Both guar gum and locust bean gum are added as a thickening agent and water-binding agent (among other roles) to products such as bakery goods, sauces, dairy products, ice creams, dips, and salad dressings. Gums are also found naturally in foods such as oatmeal, barley, and legumes. Some gums (xanthan gum and gellan gum) can be synthesized by microorganisms. Gums are considered to be both dietary and functional fibers.

β -GLUCANS

β -glucans are homopolymers of glucopyranose units (Figure 4.2f). This water-soluble dietary fiber is found in relatively high amounts in cereal brans, especially oats and barley. Oat β -glucan consists of a chain of β -D-glucopyranosyl units joined mostly in β 1-4 linkages but also some β 1-3 linkages. β -glucans, extracted from cereals, are used commercially as a functional fiber because of their effectiveness in reducing serum cholesterol and postprandial blood glucose concentrations. β -glucans are highly fermentable in the colon.

FRUCTANS—INULIN, OLIGOFRUCTOSE, AND FRUCTOOLIGOSACCHARIDES

Fructans, sometimes called *polyfructose*, including inulin, oligofructose, and fructooligosaccharides, are chemically composed primarily of fructose units in chains of varying length. Inulin consists of a fructose chain that contains from 2 to about 60 units, with β 2-1 linkages and a glucose molecule linked to the C-2 position of the terminal

fructofuranose unit to create a nonreducing unit at the end of the molecule. Although human digestive enzymes are not able to hydrolyze the β 2-1 linkage, some bacteria, such as bifidobacteria, have β -fructosidase, which can hydrolyze the β 2-1 linkage. Oligofructose is formed from the partial hydrolysis of inulin and typically contains between 2 and 8 fructose units; it may or may not contain an end glucose molecule. Fructooligosaccharides are similar to oligofructose, except that polymerization of fructooligosaccharides ranges from 2 to 4 units. Ingesting fructooligosaccharides and other fructans has been shown to promote the growth of bifidobacteria (act as a prebiotic) as discussed in more detail in the section on fermentable fibers as prebiotics.

Fructans are found naturally in plants and are considered dietary fibers, but at present they are not reported in most food composition databases. The most common food sources of inulin and other fructans include chicory, asparagus, onions, garlic, artichokes, tomatoes, and bananas. Fresh artichoke, for example, contains about 5.8 g fructooligosaccharides per 100 g, and minced dried onion flakes provide 4 g fructooligosaccharides per 100 g [3]. Wheat, barley, and rye also contain some fructans. Fructans, when added to foods, are often synthesized from sucrose by adding fructose. The fibers also can be extracted and purified from plant sources for commercial use. Inulin is used to replace fat in fillings, dressings, and frozen dessert, to name a few examples. Oligofructose is added, for example, to cereals, yogurt, dairy products, and frozen desserts. With sufficient data showing positive physiological effects, fructans added to foods could be considered a functional fiber. Americans are thought to consume up to about 4 g of fructooligosaccharides each day from foods.

RESISTANT STARCH

Resistant starch is starch that cannot be enzymatically digested, and thus absorbed, by humans. Four main types of resistant starch exist. Starch found in plant cell walls that is inaccessible to amylase activity is one type of resistant starch, designated RS₁. Food sources of RS₁ include partially milled grains and seeds. Resistant starch also may be formed during food processing. Ungelatinized granules of starch are typically resistant to enzymatic digestion and are designated RS₂. This type of starch can be found in potatoes and unripe (green) bananas. Cooking and cooling starchy foods by moist heat or extruding starchy foods, for example, generates retrograde starch called RS₃. Chemical modifications of starch, such as the formation of starch esters, or cross-bonded starches, also result in resistant starch (called RS₄). Both RS₁ and RS₂ are considered dietary fibers, whereas RS₃ and RS₄ are considered functional fibers [1]. RS₃ and RS₄ may be partially fermented by colonic bacteria. Americans consume an estimated 10 g of resistant starch daily.

CHITIN AND CHITOSAN

Chitin is an amino-polysaccharide polymer containing β 1-4 linked glucose units. It is similar in structure to cellulose, but an N-acetyl amino group substitutes for the hydroxyl group at the C-2 of the D-glucopyranose residue. Chitin is thus described as a straight chain homopolymer of N-acetyl glucosamine. Chitin can replace cellulose in the cell walls of some lower plants. It is also a component of the exoskeleton of insects and is found in the shells of crabs, shrimp, and lobsters. Chitin is insoluble in water.

Chitosan is a deacetylated form of chitin, and thus is a polysaccharide made of glucosamine and N-acetyl glucosamine. Polymers of chitosan vary in their degree of acetylation. Like chitin, chitosan has a high molecular weight, is viscous, and is water insoluble. However, lower molecular weight chitosans, manufactured through hydrolysis, are less viscous and water soluble. As a positively charged molecule in gastric juice, chitosan has the ability to interact with or complex to dietary lipids, primarily unesterified cholesterol and phospholipids, which are negatively charged. Once formed, the chitosan-lipid complex moves from the acidic stomach into the more alkaline small intestine where it forms an insoluble gel that is excreted in the feces. Consequently, the ability of chitosan supplements, about 1.4 g/day, to reduce serum cholesterol and triglyceride concentrations has been examined and shown in some, but not all, studies to be effective [4–8]. Studies in animals also suggest chitosan may augment immune system function (specifically natural killer cell activity) and minimize some adverse effects of some chemotherapeutic agents used to treat cancer [9]. With additional data showing physiological benefits in humans, chitosan and chitin may be considered functional fibers [1].

POLYDEXTROSE AND POLYOLS

Polydextrose is a polysaccharide consisting of glucose and sorbitol units that have been polymerized at high temperatures and under a partial vacuum. Polydextrose, available commercially, is added to foods as a bulking agent or as a sugar substitute. The polysaccharide is neither digested nor absorbed by the human gastrointestinal tract; however, it can be partially fermented by colonic bacteria and contributes to fecal bulk. Polyols such as polyglycerol and malitol are found in syrups. With sufficient data on physiological benefits, some polyols and polydextrose may be classified as functional fibers [1].

PSYLLIUM

Psyllium is obtained from the husk of psyllium seeds (also called plantago or fleas seed). Products containing psyllium have high water-binding properties and thus provide viscosity in solutions. Psyllium, classified as a mucilage,

has a structure similar to that of gums and is considered a functional fiber. It is added, for example, to health products such as Metamucil for its laxative properties [1]. Foods containing psyllium and that bear a health claim are required to state on the label that the food should be eaten with at least a full glass of liquid and that choking may result if the product is not ingested with enough liquid [10]. In addition, the label should state that the food should not be eaten if a person has difficulty swallowing [10].

RESISTANT DEXTRINS

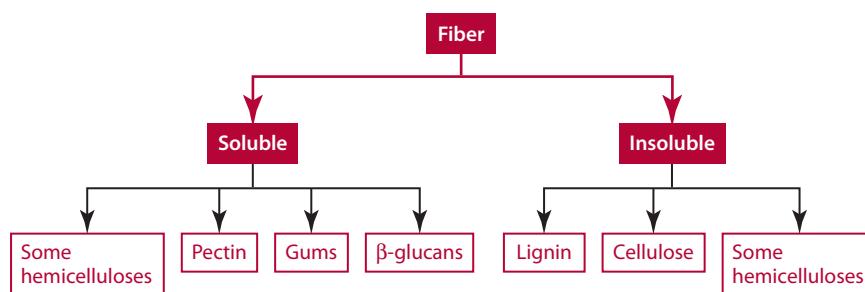
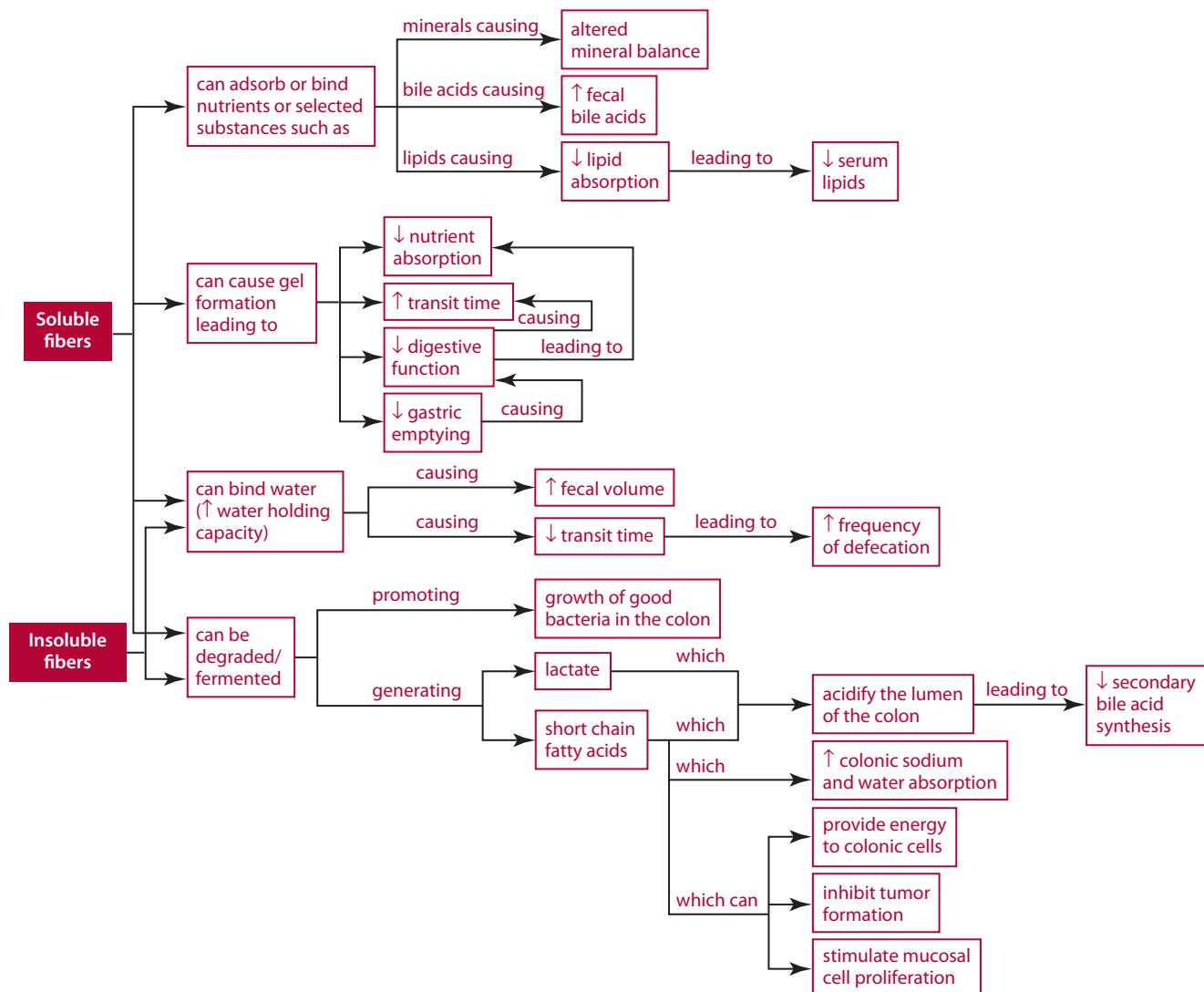
Resistant dextrans, also called *resistant maltodextrins*, are generated by treating cornstarch with heat and acid and then with enzymes (amylase). The resistant dextrans consist of glucose polymers containing α 1-4 and α 1-6 glucosidic bonds and α 1-2 and α 1-3 bonds. With sufficient data showing beneficial physiological effects, resistant dextrans may be considered functional fibers [1].

Selected Properties and Physiological and Metabolic Effects of Fiber

The physiological and metabolic effects of fiber vary based on the type of ingested fiber. Significant characteristics of dietary fiber that affect its physiological and metabolic roles include its solubility in water (as shown in Figure 4.3), its hydration or water-holding capacity and viscosity, its adsorptive attraction or ability to bind organic and inorganic molecules, and its degradability or fermentability by intestinal bacteria. The following sections review each of these characteristics and their effects on various physiological and metabolic processes. Figure 4.4 diagrams their relationships. However, as you study these characteristics and their effects on the body, remember that we eat foods with a mixture of dietary fiber, not foods with just cellulose, hemicellulose, pectins, gums, and so forth. Thus, the effects on the various body processes are not as straightforward as presented in this chapter and vary considerably based on the foods ingested.

SOLUBILITY IN WATER

Fiber is often classified as water soluble or water insoluble (Figure 4.3). Fibers that dissolve in hot water are soluble, and those that do not dissolve in hot water are insoluble. In general, water-soluble fibers include some hemicelluloses, pectin, gums, and β -glucans. Cellulose, lignin, some hemicelluloses, chitosan, and chitin are examples of

**Figure 4.3** Soluble and insoluble fibers.**Figure 4.4** Gastrointestinal response to soluble and insoluble fibers.

dietary fibers classified as insoluble. Generally, vegetables and wheat, along with most grain products, contain more insoluble fibers than soluble fibers.

Solubility in water also may be used as a basis for broadly dividing the characteristics of fibers. For example, soluble

fibers generally delay gastric emptying, increase transit time (through slower movement) through the intestine, and decrease nutrient (e.g., glucose) absorption. In contrast, insoluble fibers decrease (speed up) intestinal transit time and increase fecal bulk. These actions (discussed

in the following section) of the soluble and insoluble fibers in turn induce other physiological and metabolic effects.

WATER-HOLDING/HYDRATION CAPACITY AND VISCOSITY

Water-holding or hydration capacity of foods refers to the ability of fiber in food to bind water; think of fiber as a dry sponge moving through the digestive tract hydrating or soaking up water and digestive juices as it moves through the digestive tract. Many of the water-soluble fibers such as pectins, gums, and some hemicelluloses have a high water-holding capacity in comparison with fibers such as cellulose and lignin, which have a lower water-holding capacity. In addition, some water-soluble fibers such as pectins, psyllium, and gums form viscous (thick) solutions within the gastrointestinal tract.

Water-holding capacity, however, does not depend just on the fiber's solubility in water. The pH of the gastrointestinal tract, the size of the fiber particles, and the degree to which foods are processed also influence water-holding capacity and in turn its physiological effects. Coarsely ground bran, for example, has a higher hydration capacity than bran that is finely ground. Consequently, coarse bran with large particles holds water, increases fecal volume, and speeds up the rate of fecal passage through the colon. Maintaining the integrity of cells in grains and legumes rather than subjecting them to traditional milling processes also appears to affect the water-holding capacity of fibers. Ingesting fibers that can hold water and create viscous solutions within the gastrointestinal tract causes a number of effects:

- delayed (slowed) emptying of food from the stomach
- reduced mixing of gastrointestinal contents with digestive enzymes
- reduced enzyme function
- decreased nutrient diffusion rate (and thus delayed nutrient absorption), which attenuates the blood glucose response
- altered small intestine transit time

The following sections describe each of these effects.

Delayed (Slowed) Gastric Emptying

When fibers form viscous gels or hydrate within the stomach, the release of the chyme from the stomach (gastric emptying) into the duodenum (proximal small intestine) is delayed (slowed). Thus, nutrients remain in the stomach longer with these fibers than they would in the absence of the ingested fiber. This effect creates a feeling of postprandial (after-eating) satiety (fullness) as well as slows down the digestion process, because carbohydrates and lipids that remain in the stomach undergo no digestion in the

stomach and must move into the small intestine for further digestion to occur.

Reduced Mixing of Gastrointestinal Contents with Digestive Enzymes

The presence of viscous gels or hydrated fiber in the gastrointestinal tract provides a physical barrier that can impair the ability of the nutrients in the food to interact with the digestive enzymes. This interaction is critical for digestion to occur.

Reduced Enzyme Function

Viscous gel-forming fibers have been shown to interfere with the enzymatic hydrolysis of nutrients within the gastrointestinal tract. For example, gums may inhibit intestinal peptidases that are necessary to digest peptides to amino acids [11,12]. The activity of pancreatic lipase also has been shown to be diminished because of ingestion of viscous gel-forming fibers. The diminished lipase activity in turn inhibits lipid digestion [11]. Whether fiber directly decreases the activity of these digestive enzymes or acts by reducing the rate of enzyme penetration into the food is unclear.

Decreased Nutrient Diffusion Rate—Attenuation of the Blood Glucose Response

Remember that for nutrients to be absorbed they must move from the lumen of the small intestine through a glycoprotein-rich (i.e., mucin-rich) water layer lying on top of the enterocytes and finally into the enterocyte. The fiber-associated decreased diffusion rate of nutrients through this water layer is thought to be caused by an increased thickness of the unstirred water layer. In other words, the unstirred water layer becomes more viscous and resistant to nutrient movement, and without this movement nutrients cannot be absorbed into the enterocyte.

Another mechanism may also be responsible for decreased nutrient diffusion. Gums appear to slow glucose absorption by decreasing the convective movement of glucose within the intestinal lumen. Convective currents induced by peristaltic movements bring nutrients from the lumen to the epithelial surface for absorption. Decreasing the convective solute movement also may help explain why absorption of amino acids and fatty acids is decreased by viscous fiber [11]. Ingesting viscous fibers such as gums, pectin, β -glucans, psyllium, and to a variable extent some chitosans, fructooligosaccharides, and polydextrose, has been shown to slow transit, delay glucose absorption, lower blood glucose concentrations, and affect hormonal (especially glucagon-like peptide 1 and insulin) response to the absorbed nutrient [4,5,8,13–24]. Moreover, adding crystalline cellulose to the diets of some animals also has resulted in increased viscosity (thickness) of digestive tract contents and chyme and reduced glucose absorption secondary to diminished glucose

diffusion within the luminal contents [25]. Such effects are beneficial to people with diabetes mellitus and reduce postprandial blood glucose concentrations and insulin needs and response.

A decreased nutrient diffusion rate may in turn result in nutrients “missing” their normal site of maximal absorption. For example, if a nutrient is normally absorbed in the proximal intestine but because of gel formation is “trapped” as part of the gel, then absorption cannot occur at this site. Should the nutrient be released from the gel, the release is most likely going to occur at a site distal to where the nutrient would normally have been absorbed. The extent to which nutrients are absorbed throughout the digestive tract varies with the individual nutrient.

Altered Small Intestine Transit Time

In general, soluble fibers typically delay (slow down or increase) small intestine transit time versus insoluble fibers, which decrease (speed up or shorten) transit time within the small intestine. As with decreased diffusion rates, the changes in transit time, especially if it is shortened, may result in decreased nutrient absorption because the nutrients are in contact with enterocytes for too short a time.

ADSORPTION OR BINDING ABILITY

Some fiber components, especially lignin, gums, pectins, some hemicelluloses, and some modified forms of chitosans have the ability to bind (adsorb) substances such as enzymes and nutrients in the gastrointestinal tract. Maillard products also have this binding ability. Maillard products consist of enzyme-resistant linkages between the amino group ($-\text{NH}_2$) of amino acids, especially the amino acid lysine, and the carboxyl groups ($-\text{COO}^-$) of reducing sugars. Maillard products are formed during heat treatment, particularly in baking and frying foods, and typically cannot be digested by human digestive enzymes. The ability of some Maillard products and fibers to adsorb substances depends in part on gastrointestinal pH as well as particle size, food processing, and fermentability [26]. Ingesting fibers with adsorption properties within the gastrointestinal causes these physiological effects:

- diminished absorption of lipids
- increased fecal bile acid excretion
- lowered serum cholesterol concentrations (hypcholesterolemic properties)
- altered mineral and carotenoid absorption

The mechanisms by which these effects occur vary considerably and are reviewed next.

Diminished Absorption of Lipids

Soluble fibers (e.g., pectin, gums, and β -glucans) but also the insoluble fiber lignin and modified forms of chitosan

may affect lipid absorption by adsorbing or interacting with fatty acids, cholesterol, and bile acids within the digestive tract. Fatty acids and cholesterol that are bound or complexed to fiber cannot form micelles and cannot be absorbed in this bound form; only free fatty acids, monoacylglycerols, and cholesterol can be incorporated into micelles. Remember that micelles are needed for these end products of fat digestion to be transported through the unstirred water layers and into the enterocyte. Thus, fiber-bound lipids typically are not absorbed in the small intestine and pass into the large intestine where they are excreted in the feces or degraded by intestinal bacteria.

Increased Fecal Bile Acid Excretion

Adsorption of bile acids to fibers prevents the use of the bile acids for micelle formation. And, like fiber-bound fatty acids, bile acids bound to fiber cannot be reabsorbed and recirculated (enterohepatic recirculation). Fiber-bound bile acids typically enter the large intestine where they are degraded by colonic microflora and excreted in the feces.

Lowered Serum Cholesterol Concentrations (Hypocholesterolemic Properties)

The ability of some fibers to lower serum cholesterol concentrations is based on a series of events. First, when excretion of bile acids and cholesterol in the feces increases, less bile undergoes enterohepatic recirculation. A decrease in the bile acids returned to the liver and decreased cholesterol absorption lead to a decreased cholesterol content of liver cells. Decreased hepatic cholesterol promotes removal of LDL cholesterol from the blood. The decrease in returned bile acids to the liver also necessitates the use of cholesterol for synthesis of new bile acids. The net effect is a lower serum cholesterol. A second proposed mechanism for the hypocholesterolemic (lower blood cholesterol) effect of fiber is the shift of bile acid pools away from cholic acid and toward chenodeoxycholic acid. Chenodeoxycholic acid appears to inhibit 3-hydroxy 3-methylglutaryl (HMG) CoA reductase, a regulatory enzyme necessary for cholesterol biosynthesis [12]. Decreased HMG CoA reductase activity results in reduced hepatic cholesterol synthesis and theoretically lower blood cholesterol concentrations. A third mechanism suggests that production of propionate or other short-chain fatty acids from bacterial degradation of fiber (discussed in the next section) lowers serum cholesterol concentrations, possibly through inhibitory effects on fatty acid synthesis, cholesterol synthesis, or both [27–30]. However, propionate fed to humans has had varying effects on serum cholesterol concentrations.

Studies have demonstrated that ingesting psyllium, some gums (especially guar gum), β -glucan and oat products, resistant dextrans, and pectin can lower serum cholesterol concentrations to varying degrees [19,24,31–45].

Variable effects on blood lipid concentrations also have been observed with ingestion of inulin, fructooligosaccharides, and chitosan supplements [4–8,14,17,19,23]. Quantities of soluble fiber needed to lower serum lipid concentrations vary from about <10 g per day for pectins and gums to up to 150 g of dried beans or legumes. To consume the necessary amount of soluble fiber from foods to lower serum lipids, one would need to ingest, for example, about 6 to 10 servings per day of soluble fiber-rich fruits and vegetables, or about 2 to 3 servings per day of legumes or oat- or barley-based cereals. Ingesting foods such as corn, wheat, or rice bran, which are rich in insoluble fibers, is less effective in lowering serum lipids [22,44,45], yet diets rich in whole grains generally protect against risk for heart disease [46,47].

In addition to the ability of various fibers to lower serum cholesterol, other plant components, specifically phytostanols and sterols, also lower serum cholesterol by binding bile and dietary and endogenous cholesterol in the gastrointestinal tract and enhancing its fecal excretion from the body. Daily consumption of plant sterols and stenols in amounts ranging from about 1.6 to 3 g/day has been shown to decrease total and LDL plasma cholesterol concentrations in people with normal blood lipid and high blood lipid concentrations [48].

Altered Mineral and Carotenoid Absorption

Some fibers—especially those with uronic acid, such as hemicellulose, pectins, and gums—as well as fructose and galactose oligosaccharides can form cationic bridges with minerals within the gastrointestinal tract. Lignin, which has both carboxyl and hydroxyl groups, is also thought to play a role in mineral adsorption. The overall effect (positive or negative) that fiber has on mineral balance depends to some extent on its degree of fermentability or its accessibility to bacterial enzymes in the colon. Microbial proliferation from slowly fermentable fibers may result in increased binding of minerals within the new microbial cells and in the loss of minerals from the body, assuming colonic mineral absorption. In contrast, the more rapidly fermentable fibers (such as pectins and oligosaccharides) appear to have a favorable effect on mineral balance. The acidic environment generated by bacterial fermentation of some fibers is thought to increase mineral solubility, act with calcium to enhance activity of exchange system transporters, or both. Calcium, zinc, and iron bound to these fiber components appear to be released as fermentation occurs and may be absorbed in the colon [12].

The absorption of carotenoid and some phytochemicals also has been shown to be negatively affected by ingestion of fibers, especially pectin and guar gum. Reductions (33% to 74%) in the absorption of β-carotene, lycopene, lutein, and canthaxanthin have been demonstrated when pectin or guar gum are added to the diet.

DEGRADABILITY/FERMENTABILITY

Fiber reaches the colon undigested by human digestive enzymes. Some fiber, however, can be degraded (fermented) to varying degrees by colonic microflora. This section discusses first fermentable fibers and their effects on the body. A discussion of the effects of fibers that are not fermentable or are less fermentable follows.

Fermentable Fibers

Many fibers are fermented in the digestive tract. Those that are most fermentable include fructans, pectin, gums, psyllium, polydextrose, and resistant starch. In addition to these fibers, some cellulose and hemicelluloses are also fermentable, but their fermentation is much slower than that of the other fibers. Fermentable fibers provide many benefits to the body. For example, some fermentable fibers stimulate the production of bacteria. Fermentable fibers also can generate short-chain fatty acids for use by the body. Both of these roles are discussed hereafter.

Fermentable Fibers as Prebiotics In addition to being degraded by intestinal microflora, some fibers (but not all) have been shown to function as a prebiotic. Prebiotics serve as substrates to promote the colonic growth of selected health-promoting species of bacteria. Fibers that have been shown to function as prebiotics include inulin, oligofructose, fructooligosaccharides, pectin, β -glucans, gums, and RS₃. In addition, other partially digestible sugars such as soybean oligosaccharides, galactose oligosaccharides, and lactulose (a keto-analogue of lactose) also have been shown to promote the growth of health-promoting bacteria. Galactose oligosaccharides and soybean oligosaccharides include sugars such as raffinose, stachyose, and verbascose. Raffinose is a trisaccharide of fructose and glucose to which galactose is linked in an α 1-6 glycosidic linkage. Stachyose is similar to raffinose but has an additional galactose molecule so it is a tetrasaccharide of fructose, glucose, and galactose to which another galactose is linked. Verbascose is an oligosaccharide containing fructose, glucose, and three galactose molecules. These sugars, shown in Figure 4.5, are found in a variety of peas and beans including soybeans, chick peas, field peas, green peas, lentils, and mung, lima, snap, northern, and navy beans, among others. The fibers that function as prebiotics have been shown to stimulate the colonic growth of lactobacilli and bifidobacteria, both health-promoting bacteria. The amount of prebiotics needed to increase the colonic bifidobacteria population varies with the different fibers. Generally, ingesting about 10 to 15 g daily of inulin, oligofructose, fructooligosaccharides, galactose oligosaccharides, lactulose, or RS₃ for at least 14 to 21 days is sufficient [28,49,50]. However, because of differences in methodology, form and dose of substrate (prebiotic), duration, subjects, and types of measurements collected,

comparisons of the efficacy of different prebiotics cannot be accurately made at this time [51]. The increased presence of the beneficial bacteria has been shown to reduce the presence of pathogenic or potential pathogenic bacteria (such as *Clostridium perfringens* and salmonella) and may be useful in preventing and treating various diseases or conditions such as diarrhea [52]. See Chapter 2 for additional information on intestinal bacteria.

Short-Chain Fatty Acid Generation The principal metabolites of fermentable fibers are lactate and short-chain fatty acids, formerly called volatile fatty acids because of their volatility in acidic aqueous solutions. The short-chain fatty acids include primarily acetic, butyric, and propionic acids. Other products of fiber fermentation are hydrogen, carbon dioxide, and methane gases that are excreted as flatulence or expired by the lungs. Different fibers are fermented to different short-chain fatty acids in different amounts by different bacteria. For example, ingesting pectin resulted in higher propionate concentrations in the colons of rats

than ingesting wheat bran, which resulted in higher butyrate concentrations [53]. Furthermore, bacteroids that act on pectin generate acetate, propionate, and succinate, whereas eubacteria yield acetate, butyrate, and lactate from pectin degradation. In addition, bifidobacteria produce acetate and lactate from pectin fermentation. Some general effects of these short-chain fatty acids include:

- increased water and sodium absorption in the colon
- mucosal cell proliferation
- provision of energy
- acidification of luminal environment

The next sections briefly review each of these general effects.

Increased Water and Sodium Absorption in the Colon

Short-chain fatty acids produced by fermentation are rapidly absorbed, and their absorption in turn stimulates water and sodium absorption in the colon.

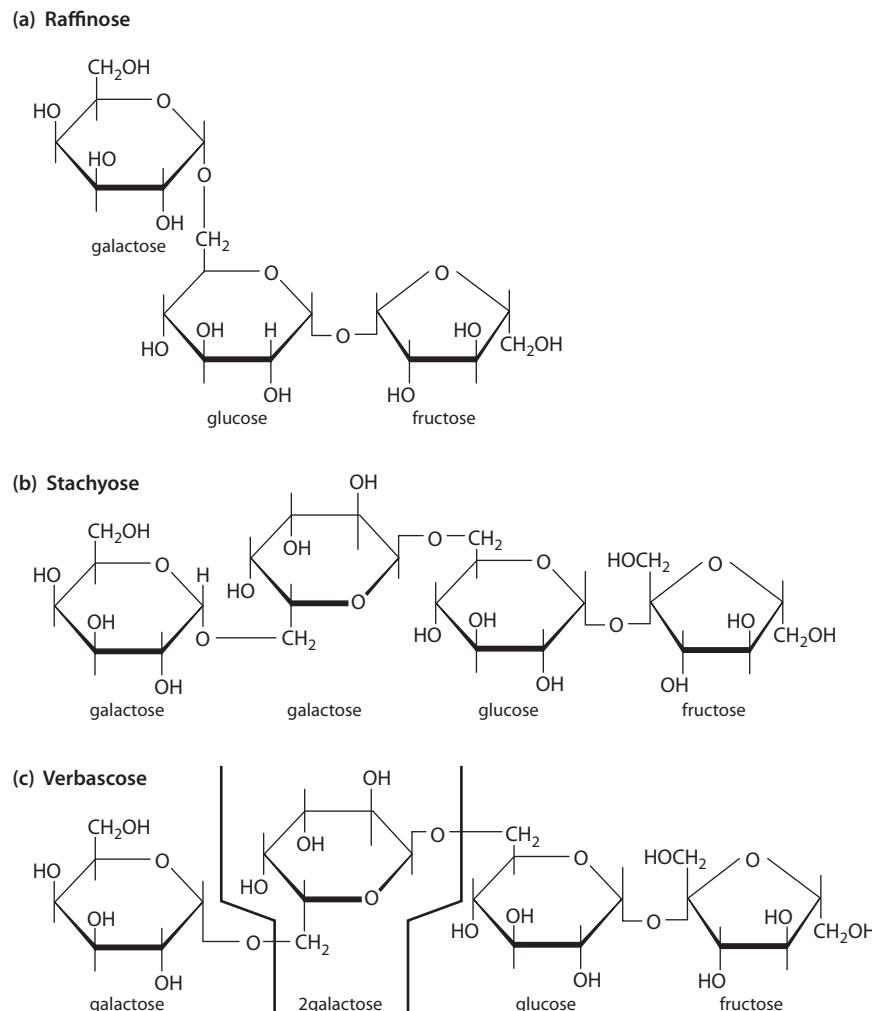


Figure 4.5 Chemical structures of some galactose oligosaccharides that may promote the growth of healthy bacteria in the gastrointestinal tract.

Mucosal Cell Proliferation Substrates generated from the degradation of dietary fiber in the colon stimulate the proliferation of mucosal cells in the gastrointestinal tract.

Provision of Energy Short-chain fatty acids provide body cells with substrate for energy production. Butyric acid provides an energy source for colonic epithelial cells. Those fatty acids not used by the colonic cells, primarily the propionic and acetic acids, are carried by the portal vein to the liver, where the propionate and some of the acetate are taken up and metabolized. Most of the acetate, however, passes to the peripheral tissues, where it is metabolized by skeletal and cardiac muscle. Remember from the section on lowered serum cholesterol that the propionic acid generated from fiber fermentation can inhibit hepatic cholesterol biosynthesis in rats.

Fermentation of carbohydrates by colonic anaerobic bacteria makes available to the body some of the energy contained in undigested food reaching the cecum. The exact amount of energy realized depends mostly on the amount and type of dietary fiber that is ingested. In developed countries, as much as 10% to 15% of ingested carbohydrate may be fermented in the colon; in the third-world (developing) countries, this percentage may be considerably higher [54].

Acidification of Luminal Environment The generation of short-chain fatty acids in the colon from bacterial fiber fermentation results in a decrease in the pH of the colon's luminal environment. With the more acidic pH, free bile acids become less soluble. Furthermore, the activity of bacterial 7 α dehydroxylase diminishes (optimal pH ~6–6.5) and thus decreases the rate of conversion of primary bile acids to secondary bile acids. With the lower pH, calcium also becomes more available (soluble) to bind bile and fatty acids [55]. These latter two changes may be protective against colon cancer.

Nonfermentable Fibers

Fiber components that are nonfermentable, principally cellulose and lignin, or that are more slowly fermentable, such as some hemicelluloses, are particularly valuable in promoting the proliferation of microbes in the colon. Microbial proliferation may be important both for detoxification and as a means of increasing fecal volume (bulk).

Detoxification The detoxification role is based on the theory that the synthesis of increased microbial cells (i.e., microbial proliferation) could result in the increased microbial scavenging and sequestering of substances or toxins, which eventually are excreted. Alternately, certain colonic bacteria appear to inhibit proliferation of tumor cells and delay tumor formation. In addition, bacteria

such as *Lactobacillus acidophilus* can reduce the activity of enzymes that catalyze the conversion of procarcinogens to carcinogens [56].

Increased Fecal Volume (Bulk) In addition to its detoxifying role, microbial proliferation may promote increased fecal volume or bulk. Fecal bulk consists of unfermented fiber, salts, water, and bacterial mass. In general, fecal bulk increases with increased bacterial proliferation. This increase occurs not only because of the mass of the bacteria but also because bacteria are about 80% water. Thus, with increased fecal bacteria present, mass increases, and so does the water-holding capacity of the feces.

In general, fecal bulk increases as fiber fermentability decreases. The rapidly fermentable fibers, such as pectins, gums, and β-glucans, appear to have little or no effect on fecal bulk. Wheat bran is one of the most effective fiber laxatives because it can absorb three times its weight of water, thereby producing a bulky stool. Gastrointestinal responses to wheat bran include:

- increased fecal bulk
- greater frequency of defecation
- reduced intestinal transit time
- decreased intraluminal pressure

Rice bran has been found to be even more effective than wheat bran in eliciting an increased fecal bulk and a reduced intestinal transit time; both rice and wheat bran are helpful in treating constipation [57]. Other fibers that have been shown to increase fecal bulk and decrease stool transit time to improve laxation include cellulose, psyllium, inulin, and oligosaccharides [58–62].

Roles of Fiber in Disease Prevention and Management

The importance to health of an adequate fiber intake is demonstrated by some of the physiological effects exerted by the various dietary fibers. Particularly noteworthy are the hypoglycemic and hypolipidemic effects of soluble fibers. Slowing the absorption rate of carbohydrate can help the person with diabetes mellitus in regulating blood glucose concentrations. Serum cholesterol concentrations in excess of about 200 mg/dL are considered a risk factor for heart disease. Thus, ingestion of soluble fibers that can lower serum cholesterol to acceptable concentrations is beneficial against heart disease. Adequate intake of insoluble, nonfermentable fiber also has been recognized as important in treating several gastrointestinal disorders, including diverticular disease, gallstones, irritable bowel

syndrome, and constipation. Evidence for the effectiveness of fiber in the control of other diseases appears equivocal; however, populations with higher fiber intakes have a lower incidence of gastrointestinal disorders, heart disease, and breast and colon cancers [63–80]. A generous fiber intake also appears to benefit some people in their efforts at weight control. The bulk provided by fiber may have some satiety value. High-fiber foods may reduce the hunger associated with caloric (energy) restriction while simultaneously delaying gastric emptying and somewhat reducing nutrient utilization [79–81]. The effectiveness of a high-fiber diet as a treatment for obesity remains unclear.

Although different fibers exhibit different characteristics, in some cases ingesting particular foods, and not just fiber, has been shown to influence disease risk. Ingestion of whole-grain cereals, not just cereal fiber, for example, has been shown to negatively correlate with risk of death from heart disease [46,47]. Similarly, the postprandial insulin response, which in turn influences plasma glucose concentrations, has been shown to be influenced by the form of food and botanical structure rather than by the amount of fiber or type of grain product [82].

The U.S. Food and Drug Administration has approved several health claims related to fiber. The claims typically focus on fruits, vegetables, and grain products. For example, for one claim, the food requirements include fruits, vegetables, or grain products that are low in fat and a good source of dietary fiber without fortification. An example of such a claim is “Low fat diets rich in fiber-containing grain products, fruits and vegetables may reduce the risk of some types of cancer, a disease associated with many factors” [10]. Another sample claim for fruits, vegetables, and grain products that are low in fat (saturated and total) and cholesterol and contain at least 0.6 g soluble fiber per reference amount without fortification is “Diets low in saturated fat and cholesterol and rich in fruits and vegetables and grain products that contain some types of dietary fiber, particularly soluble fiber, may reduce the risk of heart disease, a disease associated with many factors” [10]. Another model claim—“Low fat diets rich in fruits and vegetables (foods that are low in fat and may contain dietary fiber, vitamin A, or vitamin C) may reduce the risk of some types of cancer, a disease associated with many factors”—has also been approved for fruits and vegetables that are a good source of vitamin A or C or dietary fiber [10]. A fourth claim is associated with β -glucan from oat bran (containing at least 5.5% β -glucan soluble fiber), rolled oats or oatmeal (containing at least 4% β -glucan soluble fiber), whole oat flour (providing at least 4% β -glucan soluble fiber), or psyllium husk with a purity of no less than 95%. An example related to this claim is “Soluble fiber from foods such as [name of soluble fiber source and food product], as part of a diet low in saturated fat and

cholesterol, may reduce the risk of heart disease. A serving of [name of food product] supplies [amount] grams of the [necessary daily dietary intake for the benefit] soluble fiber from [name of soluble fiber source] necessary per day to have this effect” [10].

Mechanisms by which dietary fibers prevent disease are multiple and varied [1,55,67–74,78,83,84]. Some of the many mechanisms of action that have been proposed for fiber’s preventive role against colon cancer are highlighted here.

- High bile acid concentrations are associated with a high risk of colon cancer. Thus, fibers that adsorb bile acids to promote fecal excretion have a protective effect by decreasing free concentration and the availability of bile acids for conversion to secondary bile acids, which are thought to promote colon carcinogenesis.
- Fibers that increase fecal bulk decrease the intraluminal concentrations of procarcinogens and carcinogens and thereby reduce the likelihood of interactions with colonic mucosal cells.
- Provision of a fermentable substrate to colonic bacteria alters bacteria species and numbers, which may inhibit proliferation or development of tumor cells or conversion of procarcinogens to carcinogens.
- A shortened fecal transit time decreases the time during which toxins can be synthesized and in which they are in contact with the colon.
- Fiber fermentation to short-chain fatty acids decreases the interluminal pH, thereby decreasing synthesis of secondary bile acids, which have been shown to promote the generation of tumors.
- Degradation of fiber by fermentation may release fiber-bound calcium. The increased calcium in the colon may help eliminate the mitogenic advantage that cancer cells have over normal cells in a low-calcium environment.
- Butyric acid appears to slow the proliferation and differentiation of colon cancer cells.
- Insoluble fibers, such as lignin, that resist degradation bind carcinogens, thereby minimizing the chances of interactions with colonic mucosal cells.

Not all studies show anticarcinogenic effects with fiber. In some studies, soluble fibers enhance the development of colorectal cancers. Proposed mechanisms for such action include: (1) soluble fibers reduce the ability of insoluble fibers to adsorb hydrophobic carcinogens and thus more carcinogens may enter the colon maintained in solution than adsorbed onto insoluble fibers; (2) on degradation of soluble fibers, carcinogens are released and deposited on the colonic mucosal surface; (3) soluble fibers may cross the intestinal epithelium and transport with them carcinogens maintained in solution; (4) soluble fibers may

reduce absorption of bile salts and thereby increase the chance for conversion to secondary bile acids [55,67]. Little agreement exists among the numerous studies designed to determine the effect of fiber in the development of colon cancer. Most of the evidence for the positive role of fiber in colon cancer prevention has come from epidemiological observations. Unfortunately, in these epidemiological studies variation in many dietary factors other than fiber intake has been noted. The dietary factors most often identified as being involved in variations in the incidence of colorectal cancer among different population groups are too many total calories, high fat, too much protein, low fiber, low intake of vitamin D and calcium, and low intake of antioxidants [67]. Meta-analyses, however, of both epidemiological and case-controlled studies that investigated dietary fiber and colon cancer found that fiber-rich diets were associated with a protective effect against colon cancer in most studies [78,84]. Furthermore, risk of colorectal cancer in the United States is thought to be reducible by up to 31% with a 13 g daily increase in dietary fiber intake [78].

Recommended Fiber Intake

Recommendations for increasing the amount of fiber in the U.S. diet have come from several governmental and private organizations, each with a concern for improving the health of the U.S. public [84–89]. In 2002, the National Academy of Sciences Food and Nutrition Board established Dietary Reference Intakes, specifically adequate intakes (AI), for fiber. Adequate intakes of total fiber, representing the sum of dietary fiber and functional fiber, were established based on amounts of fiber shown to protect against heart disease [1]. The recommendations for fiber intake for adults and children are shown Table 4.2.

No Tolerable Upper Intake Level for dietary fiber or functional fiber has been established [1].

Dietary changes encouraged in order to accomplish an increased fiber intake are consistent with the United States Department of Agriculture's MyPyramid [90], which encourages people to consume:

- fiber-rich legumes
- at least 4½ cups of fruits and vegetables per day
- at least 3 servings per day of whole grains

Table 4.2 Recommended Fiber Intakes

Population Group	Age (years)	Total Fiber (g)
Men	19 to 50	38
	≥ 51	31
Women	19 to 50	25
	≥ 51	21
Children	1 to 3	19
	4 to 8	25
Girls	9 to 18	26
Boys	9 to 13	31
	14 to 18	38

Notice that recommendations that fiber intake be increased are interpreted in terms of dietary change rather than addition to the diet of fiber supplements, which more than likely are devoid of other nutrients. As a person incorporates high-fiber foods, the percentage of complex carbohydrates increases in relation to the amount of fat and protein in the diet, making an increase in fiber almost inevitable. It remains important, however, to eat a variety of cereals, legumes, fruits, and vegetables so that variety in dietary fibers is maximized.

Table 4.3 shows the dietary fiber content of selected foods. A quick method for calculating typical dietary fiber intakes enables assessment in a clinical setting from a food history, 24-hour diet recall, or food record without using tables or computerized diet analysis programs. Because fiber-rich foods consist primarily of fruits, vegetables, grains, legumes, nuts, and seeds, the number of servings from each of these groups can be multiplied by the mean total fiber content of each food group [91]. For example, numbers of servings (size determined from the U.S. Department of Agriculture data or food label) of fruits (not including juices) and vegetables are each multiplied by 1.5 g. The 1.5 g represents the average amount of dietary fiber per serving of fruit and per serving of vegetable. Numbers of servings of refined grains are multiplied by 1.0 g, and numbers of servings of whole grains are multiplied by 2.5 g. The totals from each of the four categories are summed and added to food-specific fiber values for legumes, nuts, seeds, and concentrated fiber sources; food-specific fiber values are obtained from databases [91]. The values calculated using this quick method were within 10% of the results obtained by looking up each individual food's fiber content [91].

Table 4.3 Dietary Fiber Content of Selected Foods

Food Group	Serving Size	Fiber (g)	Food Group	Serving Size	Fiber (g)			
Fruits (raw)								
Apple with skin	1	3.3	Asparagus	4 spears	1.2			
Banana	1	3.1	Broccoli	1 c	5.1			
Blueberries	1 c	3.5	Carrots	1 c	4.8			
Grapes	1 c	1.4	Celery	1 c	2.4			
Mango	1 c	3.0	Collards	1 c	5.3			
Orange	1	3.4	Corn	1 c	2.1			
Peach	1	1.5	Mushrooms	1 c	3.7			
Pear	1	5.1	Potato baked					
Pineapple	1 c	2.2	with skin	1	4.4			
Plum	1	0.9	boiled, no skin	1	2.4			
Strawberries	1 c	3.3						
Watermelon	1 c	0.6	Grain and Grain Products					
Legumes/Beans (cooked)								
Black	1 c	15.0	Rice (cooked)					
Kidney	1 c	16.4	white	1 c	0.6			
Lima	1 c	11.6	brown	1 c	3.5			
Navy	1 c	19.1	Bread					
Pinto	1 c	15.4	white	1 slice	0.6			
Nuts								
Almonds	1 oz	3.3	whole grain	1 slice	1.9			
Cashews	1 oz	0.9	Cereals (cold)					
Pecans	1 oz	2.7	All Bran	½ c	8.8			
Peanuts	1 oz	2.3	Raisin Bran	1 c	5.0			
Walnuts	1 oz	1.9	Corn Flakes	1 ½ c	0.8			
			Oat bran (cooked)	1 c	5.7			
			Saltine crackers	4	0.4			
			Wheat crackers	4	1.7			

Source: www.ars.usda.gov and <http://huhs.harvard.edu>

SUMMARY

Definitions have now been established for dietary and functional fibers. Dietary fibers are nondigestible carbohydrates and lignin that are intact and intrinsic in plants. Examples of dietary fibers include cellulose, hemicellulose, lignin, pectin, gums, β -glucans, fructans, and resistant starches. Functional fibers are nondigestible carbohydrates that have been isolated, extracted, or manufactured; they have been shown to have beneficial physiological effects in humans. Note that functional fibers, unlike dietary fibers, do not have to be intact or intrinsic only to plants. Functional fibers shown to have beneficial effects are cellulose, pectins, gums, β -glucans, psyllium, and resistant starches. Other fibers, including

lignin, fructans, chitin, chitosan, polydextrose, polyols, and resistant dextrins, require additional studies.

The physiological effects of fiber in the gastrointestinal tract are as varied as the number of fiber components and are determined to a large extent by the types and amounts present.

Some of the many characteristics of dietary and functional fibers shown to be beneficial include water-holding/hydration capacity and viscosity, adsorption or binding ability, and degradation/fermentability. To obtain fiber through the diet, food sources of fiber need to be varied and complementary. Assurance of a good intake of fiber requires consumption of a variety of high-fiber foods including whole-grain cereals and breads, legumes, fruits, and vegetables.

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Phytochemicals and Herbal Supplements in Health and Disease

Over the past decade, the market for herbal supplements has exploded, with more than 500 herbs marketed in the United States and sales in excess of \$3 billion/year [1]. The public's enthusiasm stems from a number of benefits purported to be derived from the use of various herbs (plants or particular parts of a plant such as the leaf, stem, root, bark, seed, flower, etc.). The top seven selling herbs in America include ginkgo biloba, St. John's wort, ginseng, garlic, echinacea, saw palmetto, and kava [2]. Benefits derived from the use of these herbs range from effects on the brain that are thought to improve memory or treat depression to treatments for the common cold. This perspective reviews some of the active ingredients—that is, the phytochemicals—in herbs (as well as in many other plant foods) and provides an overview of some of the more common herbal supplements used to maintain health and treat disease.

Phytochemicals

Phytochemicals consist of a large group of nonnutritive compounds that are biologically active in the body. As implied by the name, phytochemicals are found in plants, including fruits, vegetables, legumes, grains, herbs, tea, and spices. Of the tens of thousands of phytochemicals, polyphenolic phytochemicals make up the largest group. Dietary intake of polyphenols is estimated at about 1 g per day.

The polyphenols, which include more than 8,000 compounds, can be divided into a variety of classes depending on the classification system. One of the largest of these classes is the flavonoids, which includes several subclasses—flavonols, flavanols, flavones, flavanones, anthocyanidins, and isoflavones—listed in Table 1 along with examples of foods rich in flavonoids. The main flavonols include quercetin and kaempferol. These flavonols are widely found in foods, but the best sources of quercetin and kaempferol are onions, kale, leeks, broccoli, apples, blueberries, red wine, tea, and the herb ginkgo biloba. Flavanols are typically categorized based on chemical structure. Monomer forms are called catechins, and condensed forms are called proanthocyanins or tannins. Tannins provide astringent properties to foods and beverages. Flavanol intake has been estimated at 20 to 60 mg per day in the United States [3, 4]. Another category of flavonoids are flavones such as luteolin and apigenin; only a few foods, parsley and some cereals, have been identified as rich sources of these flavones. The flavanones, another subgroup of the flavonoids, also consist of just a few compounds: glycosides of naringenin in grapefruits,

eriodictyol in lemons, and hesperetin in oranges. A glass of fruit juice such as orange juice is thought to provide about 40 to 140 mg flavanone glycosides. Another flavonoid group is the anthocyanidins, the aglycone (unconjugated) form of anthocyanins. Anthocyanins are plant pigments found mostly in the skin of plants. Anthocyanins provide color (usually red, blue, or purple) to many fruits and vegetables. A 100 g serving of berries can provide up to 500 mg anthocyanins [3]. A final category of flavonoids listed in Table 1 is the isoflavones, which are found mostly in legumes, especially soybeans and its products. Isoflavones, along with lignans (found in seeds, whole grains, nuts, and some fruits and vegetables) and coumestans (found in broccoli and sprouts) are phytoestrogens. The main two plant isoflavones are genistein and daidzein.

Although the flavonoid group includes many of the thousands of phytochemicals, many other phytochemicals also may be found in foods. Some additional phytochemicals are listed in Table 2 along with some of their food sources. Some of these additional phytochemicals include phenolic acids, carotenoids (see also Chapter 10), terpenes, organosulphides, phytosterols, glucosinolates, and isothiocyanates, among others. Interest in the phenolic acid category has grown with the rise in coffee consumption in the United States. Coffee is a rich source of phenolic acids that are typically categorized as either derivatives of hydroxybenzoic acid or hydroxycinnamic acid. Caffeic, ferulic, p-coumaric, and sinapic acids make up the main dietary hydroxycinnamic acids. Caffeic and ferulic acids are thought to be more commonly consumed than the others, and estimated intake of the two acids is thought to be between 500 and 1,000 mg per day, especially among coffee drinkers [3]. Hydroxycinnamic acids also are found in other foods including vegetables, grains (outer layers), and fruits (especially blueberries, tomatoes, kiwis, plums, cherries,

apples). Examples of hydroxybenzoic acids include ellagic and gallic acids, which are found in especially high concentrations in red wine, tea, nuts, and berries.

Although Tables 1 and 2 list examples of foods containing some phytochemicals, note that most plant foods contain multiple phytochemicals. Tomatoes, for example, may contain as many as 10,000 different phytochemicals. In addition, the phytochemicals' composition and the phytochemicals' digestibility and absorbability can vary with the plant species, climate or environmental conditions in which the plant was grown, the plant's stage of ripeness, and the methods of storing and processing the plant, among other factors.

Most phytochemicals are found in foods in a variety of forms, and these forms influence the digestion and the absorption of the phytochemical. One common form of polyphenols in foods is as a glycoside conjugate. Some glycosides must be digested to aglycones (unconjugated forms) before being absorbed. Other phytochemicals are thought to be absorbed from the small intestine without extensive digestion. Further, many phytochemical glycosides are neither digested or absorbed in the small intestine. The mechanisms for the absorption of most phytochemicals are thought to involve a carrier; however, the complete absorptive processes have not been elucidated. Some phytochemicals that are not absorbed in the small intestine have been shown to undergo microbial degradation by colonic microflora. The bacteria hydrolyze the glycosides generating aglycones, which may undergo further metabolism to form various aromatic acids. Some of these acids, in turn, may be absorbed from the colon.

Once absorbed, most polyphenolic metabolites are conjugated in the small intestine or liver. Conjugation most often involves methylation, sulfation, or glucuronidation. These conjugated metabolites are then transported in the blood bound to plasma proteins, like albumin. The amount of the metabolites present

Table 1 Flavonoid Phytochemicals and Their Sources [3–8]

Flavonoid Subclass	Phytochemicals	Sources
Flavonols	Quercetin, kaempferol, myricetin	Onions, tea, olive, kale, leaf lettuce, cranberry, tomato, apple, turnip green, endive, ginkgo biloba
Flavanols	Catechins, epicatechins	Green tea, pear, wine, apple
Flavones	Apigenin, luteolin	Parsley, some cereals
Flavanones	Tangeritin, naringenin, hesperetin, hesperedin	Citrus fruits
Anthocyanidins	Cyanidin	Berries, cherries, plums, red wine
Isoflavones	Genistein, daidzein, equol	Legumes, especially soybeans, nuts, milk, cheese, flour, tofu, miso, soy sauce

in the plasma varies considerably with the type of polyphenolic phytochemical consumed, the food source, and the amount ingested, but little is known about the metabolism of all the different polyphenols in the body, and thus about what metabolites are present in the plasma after consumption of a specific polyphenol.

These differences in the metabolism of these thousands of phytochemicals in the body complicate the interpretation of research studies and the ability to make recommendations. Most studies have been done *in vitro*, in cultured cells, or in isolated tissues using specific glycosides or aglycone forms of the various phytochemicals. The forms of the polyphenolic phytochemicals used in the studies, however, have not been the same as the forms in which the polyphenolic phytochemicals are found in the body. Moreover, the amounts or concentrations of the phytochemical used in the studies have often been much higher than the amounts of the phytochemicals found naturally in the body.

Despite the problems with many of the studies, phytochemicals are strongly believed to play several important roles in the body. Flavonoids, for example, are found in cell membranes between the aqueous and lipid bilayers, where they exhibit antioxidant functions in the body. Specifically, flavonoids can scavenge free radicals such as hydroxyl, peroxyl, alkyl peroxyl, and superoxide and can terminate chain reactions (see the Perspective in Chapter 10 for a discussion of free radicals and

termination). Two characteristics determine whether a flavonoid is a good antioxidant: first, its ability to donate a hydrogen atom from its phenolic hydroxyl group to the free radical (similar to vitamin E), and second, the ability of its phenolic ring to stabilize the unpaired electron. Antioxidant activity is also exhibited by polyphenols other than flavonoids and by lignans (found in a variety of plant foods), carotenoids (found in brightly colored fruits and vegetables), and by resveratrol (found in grapes and peanuts).

In addition to antioxidant roles, some lignans and isoflavones exhibit antiestrogenic effects. Phytoestrogens such as the isoflavones are structurally similar to estrogen in that the phenol ring can bind to estrogen receptors on body cells. Soy products, rich in isoflavones, have been marketed for use by women during perimenopause to help alleviate some of the side effects of diminished natural estrogen in the body. In addition, the isoflavone genestein, along with lignans and some other flavonoids, has been shown to inhibit tumor formation and proliferation. Thus, teas, especially black and green teas that are especially rich in flavonoids, are enjoying increased popularity. Glucosinolates and isothiocyanates, along with terpenes and some phenolic acids such as hydroxycinnamic acid, also appear to have some protective effects against cancer (tumor formation), and phytosterols and isoflavones exhibit cholesterol-lowering effects that may be protective against heart disease. In fact, soy

products rich in isoflavones and margarines with added phytosterols are being marketed for use in the diets of people with hypercholesterolemia. Although much additional research is needed, studies strongly suggest possible roles for phytochemicals in the prevention of cardiovascular disease, cancers, and osteoporosis.

Herbs are also rich sources of many phytochemicals. They have been used for decades, and sometimes centuries, in Asia and other parts of the world to prevent and treat a variety of health problems. The purported benefits of six commonly used herbs—echinacea, garlic, ginkgo biloba, ginseng, milk thistle, and St. John's wort—are discussed in the following sections.

Echinacea

Echinacea is derived from a native North American plant species characterized by spiny cone flowering heads, similar in appearance to the daisy. Three types of echinacea, *Echinacea angustifolia*, *E. pallida*, and *E. purpurea*, are available commercially. Several parts of the plant, including the roots, flowers (tops), or leaves either alone or in combination, are used in the preparation of dietary echinacea supplements. Liquid alcohol-based extracts of the roots (*E. angustifolia*) or the expressed juice of the aerial portion (*E. purpurea*) are commonly sold forms of the herb.

Several active ingredients are found in echinacea. Some of these are thought to include high-molecular-weight polysaccharides (such as heteroxylans, arabinogalactans, and rhamnogalactans), glycoproteins, flavonoids, alkamides (such as isoburylamide), alkenes, alkynes (such as polyacetylenes), and phenolic acids (caffeic and ferulic acid derivatives such as cichoric acid, echinacosides, and cynarin). One or more of these active ingredients are thought to stimulate components of the immune system. Examples of positive effects on the immune system include increased production of cytokines by macrophages, activation of T-cells, and increased phagocytic activity, among others. Because of these actions, the herb typically is recommended for treating colds, upper respiratory infections, or flulike symptoms. Its topical application also is recommended as an aid in the healing of superficial wounds as well as for psoriasis and eczema. Meta-analyses and reviews of studies examining the effectiveness of echinacea in treating colds and upper-respiratory infections continue to suggest promising but inconclusive results.

Daily doses of the herb usually consist of 500 to 1,000 mg of ground herb or root in tablets or capsules or brewed as a tea, or 0.5 to 4 mL of liquid extracts or tinctures (depending on strength). Dosages are

Table 2 Phytochemicals and Their Sources [3–8]

Phytochemical class	Phytochemicals	Sources
Carotenoids	β-carotene, α-carotene, lutein, lycopene	Tomato, pumpkin, squash, carrot, watermelon, papaya, guava
Terpenes	Limonene, carvone	Citrus fruits, cherries, ginkgo biloba
Organosulphides	Diallyl sulphide, allyl methyl sulphide, S-allylcysteine	Garlic, onions, leeks, cruciferous vegetables: broccoli, cabbage, Brussels sprouts, mustard, watercress
Phenolic acids	Hydroxycinnamic acids: caffeic, ferulic, chlorogenic, neochlorogenic, curcumin Hydroxybenzoic acids: ellagic, gallic	Blueberry, cherry, pear, apple, orange, grapefruit, white potato, coffee bean, St. John's wort, echinacea Raspberry, strawberry, grape juice
Lignans	secoisolariciresinol, matairesinol	Berries, flaxseed/oils, nuts, rye bran
Saponins	Panaxadiol, panaxatriol	Alfalfa sprouts, potato, tomato, ginseng
Phytosterols	β-sitosterol, campesterol, stigmasterol	Vegetable oils (soy, rapeseed, corn, sunflower)
Glucosinolates	Glucobrassicin, gluconapin, sinigrin, glucoiberin	Cruciferous vegetables: broccoli, cabbage, Brussels sprouts, mustard, watercress
Isothiocyanates	Allylisothiocyanates, indoles, sulforaphane	Cruciferous vegetables (see above)

generally divided and ingested two to three times daily. A consecutive maximum limit of echinacea use of 6 to 8 weeks has been suggested, with use for 10 to 14 days thought to be sufficient, as the effectiveness of echinacea is thought to diminish over time. Major side effects from the use of echinacea have not been reported; however, allergic reactions may occur. Echinacea use by people with systemic or immune system dysfunction disorders is contraindicated.

Garlic

Garlic (*Allium sativum*) is part of the *Liliaceae* family, belonging specifically to the genus *Allium*. Garlic is similar to onions (also members of the *Liliaceae* family), chives, and leeks; all contain derivatives of the sulfur-containing amino acid cysteine, mainly S-allyl-L-cysteine sulfoxide, also known as *alliin*. *Alliin* (an odorless compound) may be converted to allicin (diallyl disulfide-S-oxide) in the presence of *alliinase*, which is exposed when garlic cells are destroyed by cutting or chewing, for example. Allicin degrades to diallyl disulfide, the main component in the odor of garlic, and to ajoene. Allicin-free components from garlic such as aged garlic extract has been identified and also linked with health benefits.

These active components of garlic and garlic extracts (classified as organosulphides) have been shown in various studies to be antilipidemic, antithrombotic, antihypertensive, anticarcinogenic, antiglycemic, antioxidant, and immune-enhancing. Meta-analyses of controlled clinical trials suggest (at present) small benefits of garlic primarily as a modest blood lipid-lowering agent (total and LDL cholesterol and triglycerides) and as an antithrombotic agent.

Garlic is available naturally (in bulbs) or in more concentrated form as tablets or capsules.

Typical daily dosages of garlic used in the studies ranged from about 600 to 900 mg of powdered garlic per day, or pills standardized to 0.6% to 1.3% allicin, equivalent to about 1.8 to 2.7 g or one-half to one clove of fresh garlic. Standardization, however, should not be based on allicin. In fact, commercial garlic preparations often fail to contain garlic's active compounds. Garlic cloves, for example, contain about 0.8% *alliin*; however, little *alliin* may be retained in the making of garlic powder. Losses of allicin to varying degrees also occur. Side effects most commonly associated with garlic consumption include unpleasant body and breath odor, heartburn, flatulence, and other gastrointestinal tract problems. People taking aspirin or on anticoagulant

therapies typically should avoid eating large amounts of garlic.

Ginkgo Biloba

Ginkgo biloba, from the *Ginkgoaceae* family, is a tall, long-living (as long as 1,000 years), deciduous tree with gray-colored bark. The tree is native to China and was introduced into North America in the 18th century. The fan-shaped leaves of *ginkgo biloba* are cultivated, formulated, and concentrated into an extract containing the active compounds. Pharmacologically active flavonoids found in the leaves include, for example, tannins and their glycosides. Some flavonoid glycosides and glucosides are quercentin and kaempferol 3-rhamnosides and 3-rutinosides. Terpenes, which are also active, consist of ginkgolides A, B, C, J, and M and bilobalides. Active ingredients in *ginkgo biloba* have been shown to induce peripheral vasodilation, reduce red blood cell aggregation and platelet activating factor, alter neurotransmitter receptors and levels, and exhibit antioxidant actions.

Ginkgo biloba is purported to improve arterial and venous blood flow, especially cerebral and peripheral vascular circulation, and to improve neurosensory function. Thus, the herb is often recommended for conditions in which poor circulation is a factor, such as intermittent claudication (pain, cramping, and fatigue in the leg muscles, which is usually a symptom of peripheral arterial occlusive disease), memory impairment, dementia, vertigo (dizziness), and tinnitus (ringing in the ears). Conclusions of meta-analyses and extensive reviews of studies examining the efficacy of *ginkgo biloba* are varied. For example, some, but not all, controlled clinical studies suggest that *ginkgo* extracts are more effective than a placebo in treating some cognitive disorders, some cerebral disorders, intermittent claudication, and vertigo; however, further clinical trials are needed. Generally, use of *ginkgo biloba* for cerebral insufficiency and memory impairment in the elderly and to treat intermittent claudication has produced promising results, but the data are not considered to be fully convincing. The herb has not been found to promote neurologic recovery after ischemic stroke.

Typical daily dosages of standardized *ginkgo biloba* extracts used in most clinical studies totaled about 120 mg (although higher dosages have been used). The *ginkgo biloba* dosages generally are divided and administered three times daily. *Ginkgo biloba* extracts typically are standardized to contain 22% to 27% flavonoids and 5% to 7% terpene lactones, of which

2.8% to 3.4% are ginkgolides and 2.6% to 3.6% are bilobalides. Benefits may not be observed for at least 6 weeks. Side effects that may be associated with the use of *ginkgo biloba* include headache, dizziness, palpitations, and mild gastrointestinal distress (nausea and abdominal pain). Contact with the whole plant may be associated with an allergic skin reaction. Interactions have been reported between *ginkgo biloba* and aspirin, and warfarin (anticoagulant).

Ginseng

Ginsengs are derived from several different species of the genus *Panax*. Both Asian (*P. ginseng*) and American (*P. quinquefolium*) ginsengs are derived from the genus *Panax*. However, these species should not be confused with Siberian or Russian ginsengs or with Brazilian and Indian ginsengs, which are derived from a different plant. Ginsengs are perennial shade plants native to Korea and China. Ginseng has been used as an herbal remedy in Asia for centuries. All parts of the plant contain pharmacologically active components; however, the root is the most often used portion of the herb. The active components of ginseng (roots) are a group of triterpenoid saponin glycosides collectively called ginsenosides. Two saponins in ginseng are panaxadiol and panaxatriol (panaxans).

Ginseng is purported to reduce fatigue and improve stamina and well-being and thus to serve as an energy enhancer, or adaptogen. In other words, ginseng is said to increase the body's ability to resist or cope with stress and to help the body build vitality. In addition, it has been suggested that ginseng is anticarcinogenic and may function as an antioxidant. Although a few studies have demonstrated that ginseng modulates some central nervous system activities to enhance performance, improve mood, diminish fatigue, and improve reaction time, among other effects, results of numerous other studies and meta-analyses assessing the effects of ginseng on the body do not support the claims. In other words, the efficacy of ginseng has not been clearly established for any indication.

Recommended dosages of ginseng (as tablets) range from about 100 to 300 mg/day to be taken in divided doses. Tinctures and fluid extracts also are available. Ginseng extracts are usually standardized to contain 4% to 7% ginsenosides. Ginseng root also is available and is used in dosages of 0.6 to 2 g. For those using ginseng, daily use for a short duration is suggested. Problems with product quality are common; despite label reports, products often contain negligible to no ginseng. Side effects most often observed from the use of ginseng

include headache, nausea, diarrhea, insomnia, and nervousness. Negative interactions have been reported between ginseng and warfarin (anticoagulant), the monoamine oxidase inhibitor phenelzine, and alcohol. In addition, germanium, an ingredient in some ginseng preparations, has been reported to induce resistance to diuretics.

Milk Thistle

Milk thistle (*Silybum marianum*), also called St. Mary's or Our Lady's thistle, is a tall (about 5 to 10 feet) herb with a milk sap and dark, shiny, prickly leaves with white veins. The herb is native to Europe (Mediterranean area) but also grown in California and the eastern part of the United States. Specific compounds found in the fruit in concentrations of about 1% to 4% include a variety of flavonolignans—silybin, isosilybin, dehydrosilybin, silydianin, silychristin, among others—and collectively called silymarin. The three main components are silybin, silydianin, and silychristin, with silybin being the most potent. Other active ingredients include apigenin, histamine, triamine, betaine, and others. Most commercial milk thistle products contain over a dozen of these flavonolignans among other compounds.

Fruits or seeds of milk thistle have been used for centuries to treat a variety of problems, mostly those associated with the liver, such as cirrhosis and hepatitis. Milk thistle exhibits antioxidant properties and is thought to be cytoprotective. It is thought to help the liver's resistance to toxic insults, to promote regeneration of liver cells, and to prevent damage to liver cells. Improved immune function and anti-inflammatory and antiproliferative activities also have been attributed to milk thistle. As an antioxidant, silybin combined with phosphatidylcholine as a phytosome is thought to preserve glutathione use in liver cells and has been shown in European studies to be hepatoprotective in doses up to about 360 mg. A two-year Egyptian study using silymarin alone reported no objective evidence of improvement in liver function in a group of people with hepatitis C ingesting silymarin; however, patients reported feeling better. The efficacy and effectiveness of milk thistle have not been established in the United States; however, studies are underway examining milk thistle for preventing and treating liver diseases and various cancers.

Milk thistle is sold as capsules usually providing about 140 mg silymarin. Standardized extracts of milk thistle provide 35 to 70 mg silymarin and are recommended three times daily to achieve beneficial

effects. Milk thistle is not very soluble; less than about 50% is usually absorbed unless it is made more water soluble as in the form of a phytosome. The herb appears to be fairly well tolerated; the only side effects reported include mild gastrointestinal distress and allergic reactions.

St. John's Wort

St. John's wort (*Hypericum perforatum*) is a perennial herb that produces golden yellow flowers. The flowers are typically harvested, dried, and extracted using alcohol and water. Major ingredients include naphthodianthrones (such as hypericin and pseudohypericin), phloroglucinols (such as hyperforin and adhyperforin), various flavonoids and flavonols (such as hyperoside, quercitrin, isoquercitrin, rutin, kaempferol, and biapigenin, among others), proanthocyanidins, xanthones, and phenolic acids (including caffeic acid, chlorogenic acid, and ferulic acid). Hypericin and hyperforin are thought to be the main active ingredients. Extracts of St. John's wort (wort is an Old English word for "plant") are usually standardized to provide 0.3% hypericin.

St. John's wort has been shown to inhibit neurotransmitter metabolism (especially of norepinephrine, dopamine, and serotonin), modulate neurotransmitter receptor concentrations and sensitivity, and alter neurotransmitter reuptake in the central nervous system. Oral consumption of St. John's extracts is recommended to treat depression and anxiety. Oily hypericum preparations also are used topically to relieve inflammation and promote healing of, for example, first-degree burns, hemorrhoids, or minor wounds. Numerous studies and meta-analyses of studies have shown that use of St. John's wort is more effective than a placebo and similarly as effective as many antidepressant therapies in treating mild to moderate depressive disorders. Moreover, the herb is often safer with respect to the side effects typically associated with the use of some antidepressant drug regimens.

Daily dosages of up to about 1,000 mg administered in divided doses are commonly recommended, with therapeutic regimens of 2–3 weeks needed before effects are expected. The herb is sold in tablets and capsules and in tea and tincture forms. Adverse effects associated with the use of St. John's wort may include headache, fatigue, gastrointestinal distress (nausea, abdominal pain), dizziness, confusion, restlessness, and photosensitivity. The photosensitivity occurs with high dosages or prolonged use and is manifested as dermatitis and mucous membrane inflammation with

sunlight exposure. Interactions have been reported between St. John's wort and anticoagulants, oral contraceptive (birth control) agents, theophylline (used for asthma), cyclosporin (used to diminish risk of transplant rejection), digoxin (heart contractility drug), and indinavir (antiviral drug).

Regulation of Herbal Supplements

The Dietary Supplement Health and Education Act of 1994 allows herbs and phytomedicinals to be sold as dietary supplements as long as health or therapeutic claims do not appear on the product label [9]. The act defines dietary supplements to include vitamins, minerals, herbal or botanical products, amino acids, metabolites, extracts, and other substances alone or in combination that are added to the diet [9]. Because herbal supplements need not comply with other laws, the consumer and retailer have no assurance that the herb in the supplement corresponds with the label description. Thus, the reputation of the producer becomes extremely important. Herbs may be contaminated or adulterated in the manufacturing process. Incorrect parts of the herb, such as the stem versus the root, or an incorrect stage of ripeness may be used in production of the supplement [10,11]. In other words, quality assurance of herbs and phytomedicinals is generally lacking in the United States [10–12]. The act itself or reviews of the Dietary Supplement Health and Education Act of 1994 are available for additional or more specific information [10,13,14].

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Garlic

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5

Lipids

Structure and Biological Importance

Fatty Acids

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Sterols and Steroids

Phospholipids

Glycolipids

Digestion

Triacylglycerol Digestion

Cholesterol and Phospholipid Digestion

Absorption

Transport and Storage

Lipoproteins

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Metabolism of Lipoproteins

Lipids, Lipoproteins, and Cardiovascular Disease Risk

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PERSPECTIVE:

The Role of Lipids and Lipoproteins in Atherogenesis

The property that sets lipids apart from other major nutrients is their solubility in organic solvents such as ether, chloroform, and acetone. If lipids are defined according to this property, which is generally the case, the scope of their function becomes quite broad. It encompasses not only dietary sources of energy and the lipid constituents of cell and organelle membranes but also the fat-soluble vitamins, corticosteroid hormones, and certain mediators of electron transport, such as coenzyme Q.

Among the many compounds classified as lipids, only a small number are important as dietary energy sources or as functional or structural constituents within the cell. The following classification is limited to those lipids germane to this section of the text, which deals with energy-releasing nutrients. Fat-soluble vitamins are discussed in Chapter 10.

1. Simple lipids
 - a. Fatty acids
 - b. Triacylglycerols, diacylglycerols, and monoacylglycerols
 - c. Waxes (esters of fatty acids with higher alcohols)
 - (1) Sterol esters (cholesterol-fatty acid esters)
 - (2) Nonsterol esters (vitamin A esters, and so on)
2. Compound lipids
 - a. Phospholipids
 - (1) Phosphatidic acids (i.e., lecithin, cephalins)
 - (2) Plasmalogens
 - (3) Sphingomyelins
 - b. Glycolipids (carbohydrate containing)
 - c. Lipoproteins (lipids in association with proteins)
3. Derived lipids (derivatives such as sterols and straight-chain alcohols obtained by hydrolysis of those lipids in groups 1 and 2 that still possess general properties of lipids)
4. Ethyl alcohol (though it is not a lipid per se, it does supply dietary energy, and its metabolism resembles lipid metabolism)

In the discussion of the structure and physiological function of lipids that follows, lipids have been grouped arbitrarily according to fatty acids, triacylglycerols (triglycerides), sterols and steroids, phospholipids, glycolipids, and ethyl alcohol. This grouping is more functional than structural.

Structure and Biological Importance

FATTY ACIDS

As a class, the fatty acids are the simplest of the lipids. They are composed of a straight hydrocarbon chain terminating with a carboxylic acid group. Therefore, they create within the molecules a polar, hydrophilic end and a nonpolar, hydrophobic end that is insoluble in water (Figure 5.1). Fatty acids are components of the more complex lipids, discussed in later sections. They are of vital importance as an energy nutrient, furnishing most of the calories from dietary fat.

The length of the carbon chains of fatty acids found in foods and body tissues varies from 4 to about 24 carbon atoms. The fatty acids may be saturated (SFA), monounsaturated (MUFA, possessing one carbon-carbon double bond), or polyunsaturated (PUFA, having two or more carbon-carbon double bonds). PUFAs of nutritional interest may have as many as six double bonds. Where a carbon-carbon double bond exists, there is an opportunity for either a *cis*

or a *trans* geometric isomerism that significantly affects the molecular configuration of the molecule. The *cis* isomerism form results in folding back and kinking the molecule into a U-like orientation, whereas the *trans* form has the effect of extending the molecule into a linear shape similar to that of saturated fatty acids. The structures in Figure 5.1 illustrate saturation and unsaturation in an 18-carbon fatty acid and show how *cis* or *trans* isomerization affects the molecular configuration.

The more carbon-carbon double bonds occurring within a chain, the more pronounced is the bending effect. The amount of bending plays an important role in the structure and function of cell membranes. Most naturally occurring unsaturated fatty acids are of the *cis* configuration, although the *trans* form does exist in some natural fats and oils. Most *trans* fatty acids are derived from partially hydrogenated fats and oils. Partial hydrogenation, a process commonly used in making margarine and frying oils, is designed to solidify vegetable oils at room temperature. Double bonds of *cis* orientation, not reduced by hydrogen in the process, undergo an electronic rearrangement to the *trans* form,

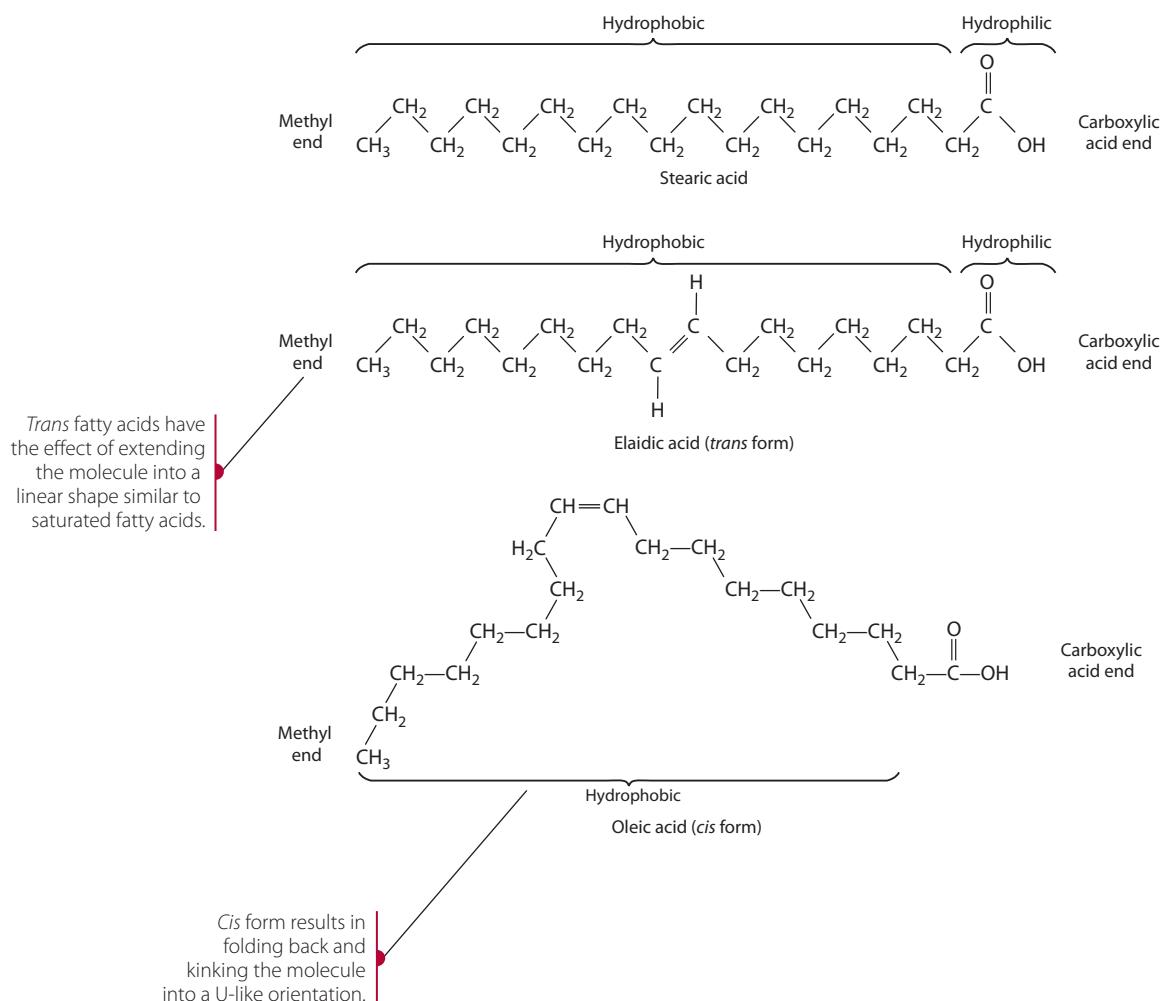


Figure 5.1 Structures of selected fatty acids.

which is energetically more stable. The availability of *trans* fatty acids in the typical U.S. diet has been estimated to be approximately 8.1 g/person/day, the major source of which is margarines and spreads [1]. The requirement to label *trans* fatty acid content of foods has encouraged manufacturers to use processes that do not result in *trans* fatty acids in the finished product and should result in lower consumption. Concerns have been raised about the possible adverse nutritional effects of dietary *trans* fatty acids, particularly their role in the etiology of cardiovascular disease. This topic is discussed in the section “Lipids, Lipoproteins, and Cardiovascular Disease Risk” in this chapter.

Fatty Acid Nomenclature

Two systems of notation have been developed to provide a shorthand way to understand the chemical structure of a fatty acid. Both systems are used regularly and will be used interchangeably in the text for different purposes.

The delta (Δ) system of notation has been established to denote the chain length of the fatty acids and the number and position of any double bonds that may be present. For example, the notation 18:2 $\Delta^{9,12}$ describes linoleic acid. The first number, 18 in this case, represents the number of carbon atoms; the number following the colon refers to the total number of double bonds present; and the superscript numbers following the delta symbol designate the carbon atoms at which the double bonds begin. In this system, the numbering starts from the carboxyl end of the fatty acid.

A second commonly used system of notation locates the position of double bonds on carbon atoms counted from the methyl, or omega (ω), end of the carbon chain. For instance, the notation for linoleic acid would be 18:2 ω -6. Substitution of the omega symbol with the letter *n* has been popularized. Using this designation, the notation for linoleic acid would be expressed as 18:2 *n*-6. In this system, the total number of carbon atoms in the chain is given by the first number, the number of double bonds is given by the number following the colon, and the location (carbon atom number) of the first double bond is given by the number following ω - or *n*-. This system of notation takes into

account the fact that double bonds in a fatty acid are always separated by three carbons. Thus, if you know the location of the first double bond, you can determine the remaining double bonds. The location of multiple double bonds is unambiguous, given their total number and the location of the one closest to either the methyl end or the carboxylic acid end, depending on the nomenclature system used.

A comparison of the two systems is shown in Figure 5.2. The designation of linoleic acid by each of the two systems is 18:2 $\Delta^{9,12}$ or 18:2 ω -6 (*n*-6). The fatty acid α -linolenic acid, which contains three double bonds, is identified as 18:3 $\Delta^{9,12,15}$ or 18:3 ω 3 (*n*-3).

Table 5.1 lists some naturally occurring fatty acids and their dietary sources. For unsaturated fatty acids, the table shows both the Δ system and the ω system. The list includes only those fatty acids with chain lengths of 14 or more carbon atoms because these fatty acids are most important both nutritionally and functionally. For example, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) together account for >90% of the fatty acids in the average U.S. diet. However, shorter-chain fatty acids do occur in nature. Butyric acid (4:0) and lauric acid (12:0) occur in large amounts in milk fat and coconut oil, respectively.

Most fatty acids have an even number of carbon atoms. The reason for this will be evident in the discussion of fatty acid synthesis. Odd-numbered-carbon fatty acids occur naturally to some extent in some food sources. For example, certain fish, such as menhaden, mullet, and tuna, as well as the bacterium *Euglena gracilis*, contain fairly high concentrations of odd-numbered-carbon fatty acids.

Essential Fatty Acids

If fat is entirely excluded from the diet of humans, a condition develops that is characterized by retarded growth, dermatitis, kidney lesions, and early death. Studies have shown that eating certain unsaturated fatty acids such as linoleic, α -linolenic, and arachidonic acids is effective in curing the conditions related to the lack of these fatty acids. Some unsaturated fatty acids thus cannot be synthesized in animal cells but must be acquired in the diet from plant foods. The

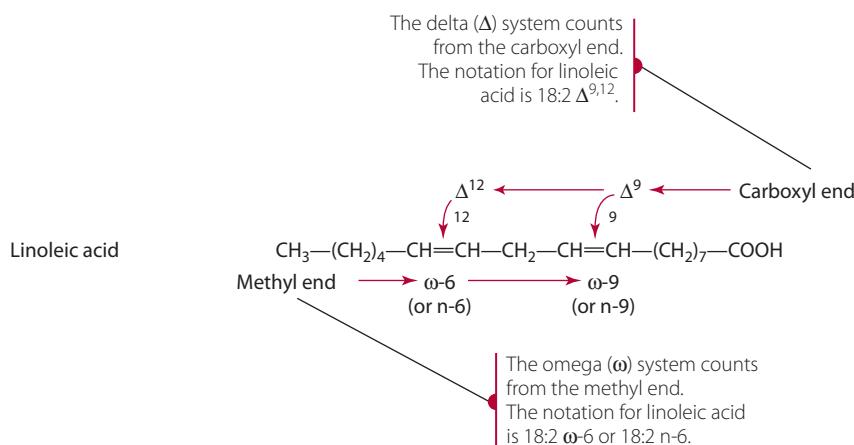


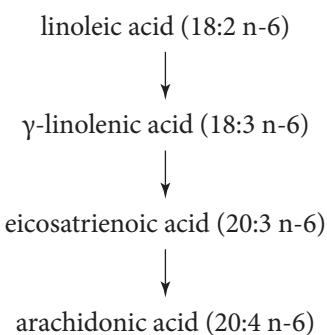
Figure 5.2 The structure of linoleic acid, showing the two systems for nomenclature.

Table 5.1 Some Naturally Occurring Fatty Acids

Notation Saturated Fatty Acids	Common Name	Formula	Source
14:0	Myristic acid	$\text{CH}_3-(\text{CH}_2)_{12}-\text{COOH}$	Coconut and palm nut oils, most animal and plant fats
16:0	Palmitic acid	$\text{CH}_3-(\text{CH}_2)_{14}-\text{COOH}$	Animal and plant fats
18:0	Stearic acid	$\text{CH}_3-(\text{CH}_2)_{16}-\text{COOH}$	Animal fats, some plant fats
20:0	Arachidic acid	$\text{CH}_3-(\text{CH}_2)_{18}-\text{COOH}$	Peanut oil
24:0	Lignoceric acid	$\text{CH}_3-(\text{CH}_2)_{22}-\text{COOH}$	Most natural fats, peanut oil in small amounts
Unsaturated Fatty Acids			
16:1 Δ^9 (n-7)	Palmitoleic acid	$\text{CH}_3-(\text{CH}_2)_5-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{COOH}$	Marine animal oils, small amount in plant and animal fats
18:1 Δ^9 (n-9)	Oleic acid	$\text{CH}_3-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{COOH}$	Plant and animal fats
18:2 $\Delta^{9,12}$ (n-6)	Linoleic acid	$\text{CH}_3-(\text{CH}_2)_4-\overset{6^*}{\text{CH}}=\overset{12^+}{\text{CH}}-\text{CH}_2-\overset{9^+}{\text{CH}}=\text{CH}-(\text{CH}_2)_7-\text{COOH}$	Corn, safflower, soybean, cottonseed, sunflower seed, and peanut oil
18:3 $\Delta^{9,12,15}$ (n-3)	α -linolenic acid	$\text{CH}_3-(\text{CH}_2-\text{CH}=\text{CH})_3-(\text{CH}_2)_7-\text{COOH}$	Linseed, soybean, and other seed oils
20:4 $\Delta^{5,8,11,14}$ (n-6)	Arachidonic acid	$\text{CH}_3-(\text{CH}_2)_3-(\text{CH}_2-\text{CH}=\text{CH})_4-(\text{CH}_2)_3-\text{COOH}$	Small amounts in animal fats
20:5 $\Delta^{5,8,11,14,17}$ (n-3)	Eicosapentaenoic acid	$\text{CH}_3-(\text{CH}_2-\text{CH}=\text{CH})_5-(\text{CH}_2)_3-\text{COOH}$	Marine algae, fish oils
22:6 $\Delta^{4,7,10,13,16,19}$ (n-3)	Docosahexaenoic acid	$\text{CH}_3-(\text{CH}_2-\text{CH}=\text{CH})_6-(\text{CH}_2)_2-\text{COOH}$	Animal fats as phospholipid component, fish oils

*Number of first carbon double bond from methyl end (n system). †Number of carbon double bond from carboxylic acid end (Δ system).

two essential fatty acids are linoleic acid (18:2 n-6) and α -linolenic acid (18:3 n-3). From linoleic acid, γ -linolenic (18:3 n-6) and arachidonic acids (20:4 n-6) can be formed in the body. An intermediate fatty acid in the pathway is eicosatrienoic acid. The pathway is



Linoleic and α -linolenic acids are essential because humans lack enzymes called Δ^{12} and Δ^{15} desaturases, which incorporate double bonds at these positions. These enzymes are found only in plants. Humans are incapable of forming double bonds beyond the Δ^9 carbon in the chain. If a $\Delta^{9,12}$ fatty acid is obtained from the diet, however, additional double bonds can be incorporated at Δ^6 (desaturation).

Fatty acid chains can also be elongated by the enzymatic addition of two carbon atoms at the carboxylic acid end of the chain. These reactions are discussed further in the “Synthesis of Fatty Acids” section of this chapter.

n-3 Fatty Acids

Nutritional interest in the n-3 fatty acids has escalated enormously in recent years because of their reported hypolipidemic and antithrombotic effects. An n-3 fatty acid of particular interest is eicosapentaenoic acid (20:5 n-3), because it is a precursor of the physiologically important eicosanoids, discussed later in this chapter. Fish oils are particularly rich in these unique fatty acids and therefore are the dietary supplement of choice in research designed to study the effects of n-3 fatty acids. Food sources and tissue distribution of a few commonly occurring n-3 polyunsaturated fatty acids are given in Table 5.2.

TRIACYLGLYCEROLS (TRIGLYCERIDES)

Most stored body fat is in the form of triacylglycerols (TAG), which represent a highly concentrated form of energy. Triacylglycerols are the currently accepted name

Table 5.2 Dietary Sources and Tissue Distribution of the Major n-3 Polyunsaturated Fatty Acids

Major Members of Series	Tissue Distribution in Mammals	Dietary Sources
α -linolenic acid 18:3 n-3	Minor component of tissues	Some vegetable oils (soy, canola, linseed, rapeseed) and leafy vegetables
Eicosapentaenoic acid 20:5 n-3	Minor component of tissues	Fish and shellfish
Docosahexaenoic acid 22:6 n-3	Major component of membrane phospholipids in retinal photoreceptors, cerebral gray matter, testes, and sperm	Fish and shellfish

that has replaced the older name triglycerides (TRIG or TG). Triacylglycerols account for nearly 95% of dietary fat. Structurally, they are composed of a trihydroxy alcohol, glycerol, to which three fatty acids are attached by ester bonds, as shown in Figure 5.3. The fatty acids may be all the same (a simple triacylglycerol) or different (a mixed triacylglycerol). Fatty acids are linked to glycerol as an ester, which liberates a water molecule during its formation. The fatty acids in acylglycerols can be all saturated, all monounsaturated, all polyunsaturated, or occur in any combination. Carbons 1 and 3 of glycerol are not the same when viewed in a three-dimensional model. Also, when different fatty acids are attached to the first and third carbons of glycerol, the second carbon becomes asymmetric. (See Chapter 3 for a discussion of stereoisomerism.) Enzymes of the body are able to distinguish between the carbons of glycerol and are generally quite specific. This specificity is important in digesting and synthesizing triacylglycerols, as will be discussed later in this chapter.

Acylglycerols composed of glycerol esterified to a single fatty acid (a monoacylglycerol, MAG) or to two fatty acids (a diacylglycerol, DAG) are present in the body in small amounts. The fatty acids can be on any of the three carbons of glycerol. However, the mono- and diacylglycerols are important intermediates in some metabolic reactions and may be components of other lipid classes. They also may occur in processed foods, to which they can be added as emulsifying agents. A diacylglycerol is on the market as a vegetable oil substitute; the manufacturer claims that using it in place of a TAG oil will result in less storage as body fat.

The specific glycerol hydroxyl group to which a certain fatty acid is attached is indicated by a numbering system for the three glycerol carbons, in much the same way as

acetaldehyde is numbered. This system is complicated somewhat by the fact that the central carbon of the glycerol is asymmetrical when different fatty acids are esterified at the two end carbon atoms and therefore may exist in either the D or the L form. A system of nomenclature called stereospecific numbering (*sn*) has been adopted in which the glycerol is always written as in Figure 5.3, with the C-2 hydroxyl group oriented to the left (L) and the carbons numbered 1 through 3 beginning at the top.

Triacylglycerols exist as fats (solid) or oils (liquid) at room temperature, depending on the nature of the component fatty acids. Triacylglycerols that contain a high proportion of relatively short-chain fatty acids or unsaturated fatty acids tend to be liquid (oils) at room temperature. Triacylglycerols made up of saturated fatty acids of longer chain length have a higher melting point and exist as solids at room temperatures. When used for energy, fatty acids are released in free (nonesterified) form as free fatty acids (FFA) from the triacylglycerols in adipose tissue cells by the activity of lipases, and the FFA are then transported by albumin to various tissues for oxidation.

STEROLS AND STEROIDS

This class of lipid is characterized by a four-ring core structure called the cyclopentanoperhydrophenanthrene, or steroid, nucleus. **Sterols** are monohydroxy alcohols of steroid structure, with cholesterol being the most common example. Cholesterol is present only in animal tissues. It can exist in free form, or the hydroxyl group can be esterified with a fatty acid. Many sterols other than cholesterol are found in plant tissues. The structure of cholesterol is shown in Figure 5.4, along with the numbering system for the carbons in the steroid nucleus and the side chain.

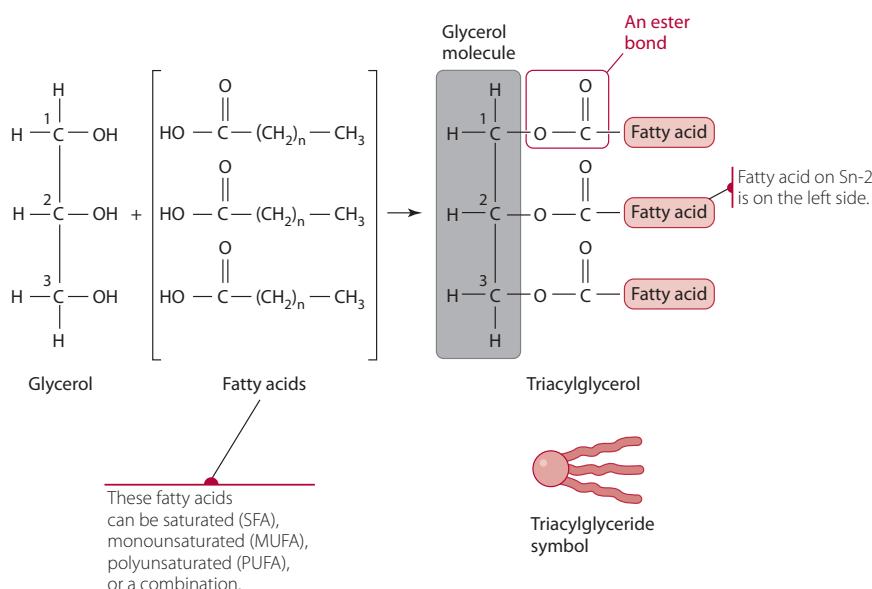
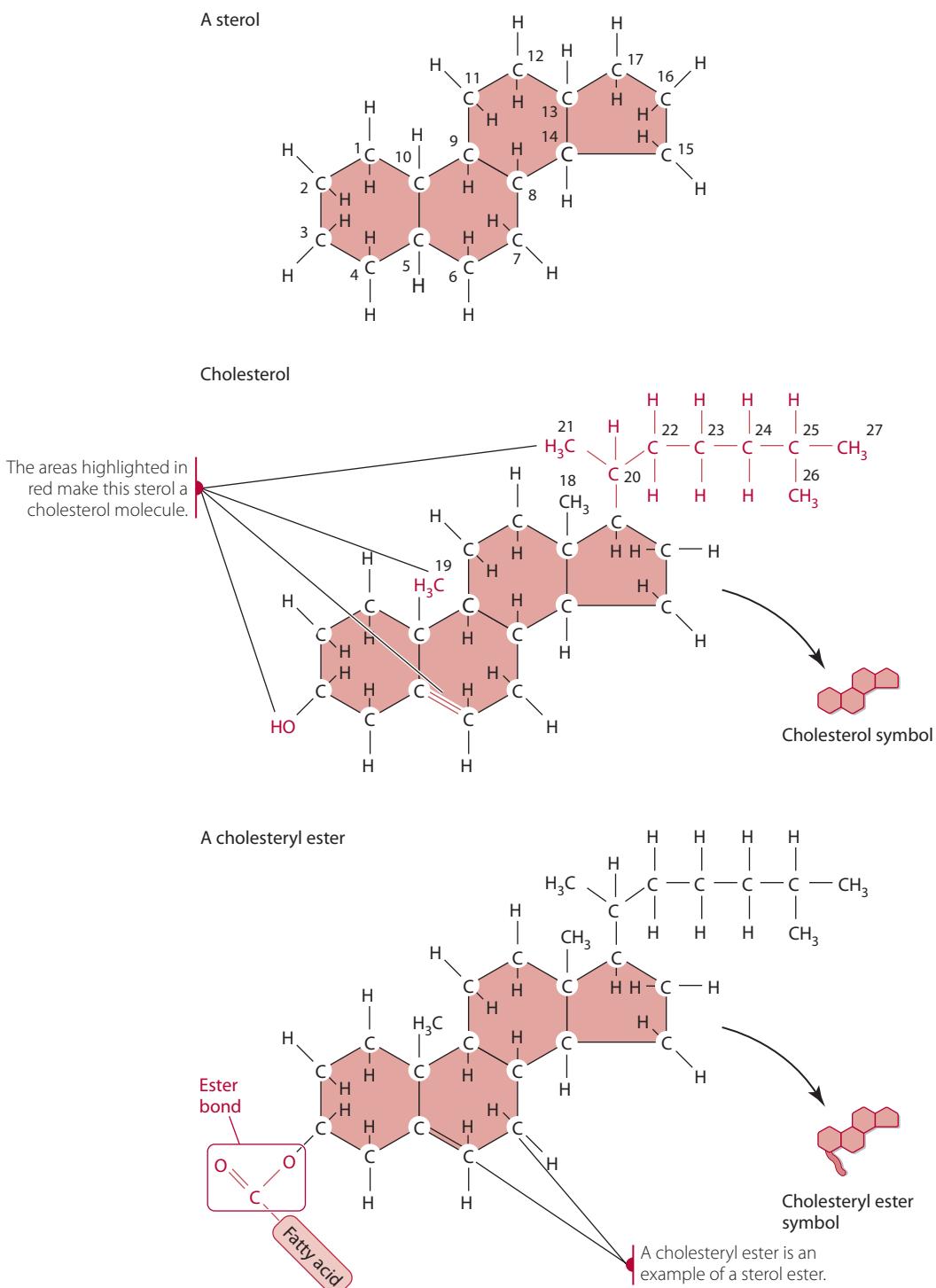


Figure 5.3 Linkage of fatty acids to glycerol to form a fatty acid.

**Figure 5.4** Structure of a sterol, cholesterol, and a cholestryly ester.

Meats, egg yolk, and dairy products, the common dietary sources of cholesterol, contain fairly large amounts. In the body, this sterol is an essential component of cell membranes, particularly the membranes of nerve tissue. Despite the bad press that cholesterol has garnered over the years because of its implication in cardiovascular disease, it serves as the precursor for many other important

steroids in the body, including the bile acids; steroid sex hormones such as estrogens, androgens, and progesterone; the adrenocortical hormones; and the vitamin D of animal tissues (cholecalciferol). These steroids differ from one another in the arrangement of double bonds in the ring system, the presence of carbonyl or hydroxyl groups, and the nature of the side chain at C-17. All these

structural modifications are mediated by enzymes that function as dehydrogenases, isomerases, hydroxylases, or desmolases. Desmolases remove or shorten the length of side chains on the steroid nucleus. The derivation of the various types of steroids from cholesterol is diagrammed in Figure 5.5. Although many physiologically active corticosteroid hormones, sex hormones, and bile acids exist, only representative compounds are shown. The biological importance and metabolic reactions of these compounds will be discussed later in this chapter.

Sterols, together with phospholipids (considered next) comprise only about 5% of dietary lipids.

PHOSPHOLIPIDS

As the name implies, lipids belonging to **phospholipids** contain phosphate. They also possess one or more fatty acid residues. Phospholipids are categorized into one of two groups called glycerophosphatides and sphingophosphatides, depending on whether their core structure is glycerol (glycerophosphatides) or the amino alcohol sphingosine (sphingolipids).

Glycerophosphatides

The building block of a glycerophosphatide is phosphatidic acid, formed by esterification of two fatty acids at C-1 and C-2 of glycerol and esterification of the C-3 hydroxyl with phosphoric acid. In most cases, glycerophosphatides have a saturated fatty acid on position 1 and an unsaturated fatty acid on position 2. The structure in Figure 5.6 typifies a phosphatidate, a term that does not define a specific structure because different fatty acids may be involved. The conventional numbering of the glycerol carbon atoms is the same as that for triacylglycerols. From top to bottom, the numbering is *sn*-1, *sn*-2, and *sn*-3, provided the glycerol is written in the L configuration so that the C-2 fatty acid constituent is directed to the left, as shown in Figure 5.6. Phosphatidic acids form a number of derivatives with compounds such as choline, ethanolamine, serine, and inositol, each of which possesses an alcohol group through which a second esterification to the phosphate takes place (Figure 5.6). The compounds are named as the phosphatidyl derivatives of the alcohols, as indicated in the figure. A common phospholipid is phosphatidylcholine, which probably is better known by

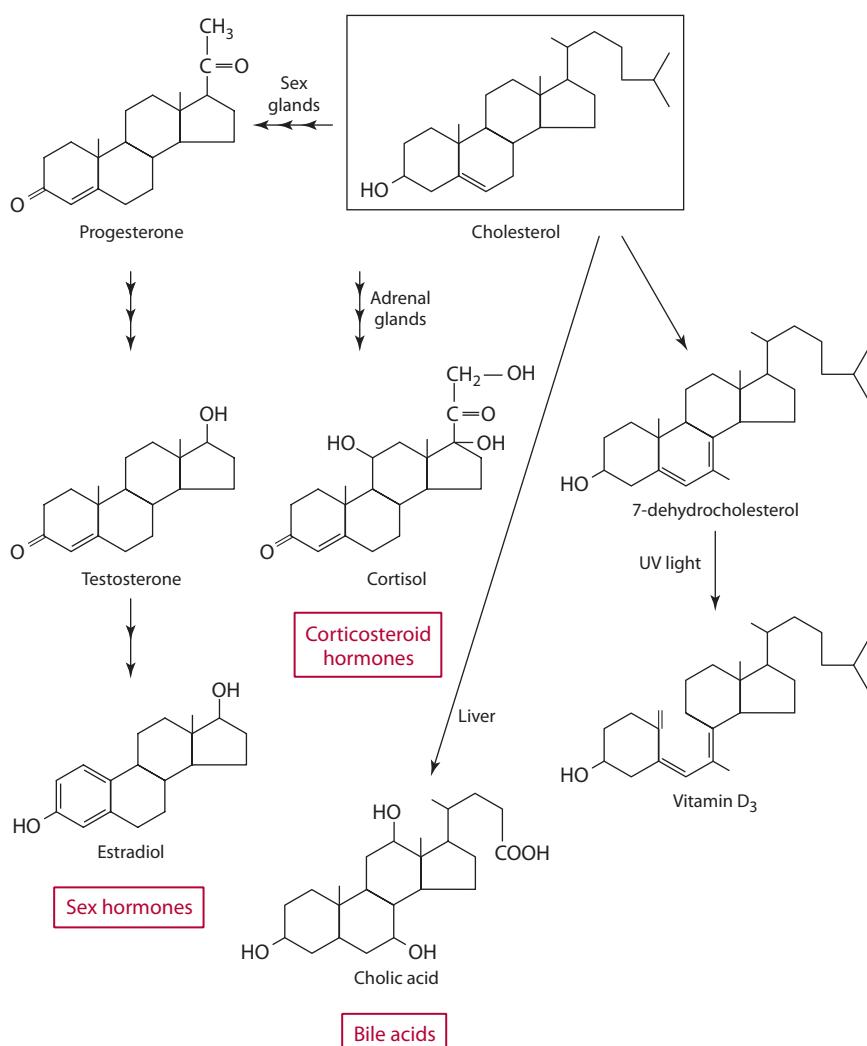


Figure 5.5 The formation of physiologically important steroids from cholesterol. Only representative compounds from each category of steroid are shown.

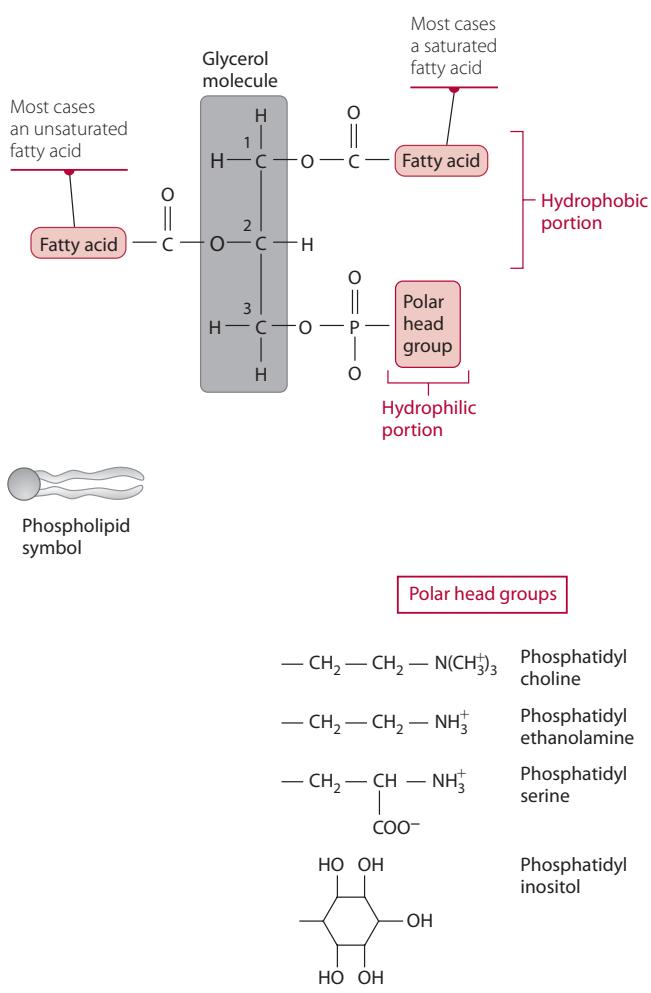


Figure 5.6 Typical structure of phospholipids.

its common name, lecithin. The other phospholipids are formed by replacing the choline in the polar head group. Phospholipids are more polar than the triacylglycerols and sterols and therefore tend to attract water molecules. Because of this hydrophilic property, phospholipids are commonly expressed on the surface of blood-borne lipid particles, such as chylomicrons, thereby stabilizing the particles in the aqueous medium. Furthermore, as a constituent of cell and organelle membranes, phospholipids serve as a conduit for the passage of water-soluble and fat-soluble materials across the membrane.

Biological Roles of Phospholipids

Phospholipids play several important roles in the body. Glycerophosphatides, for example, are very important components of cell membranes. In addition to lending structural support to the membrane, they serve as a source of physiologically active compounds. We will see later how arachidonate can be released on demand from membrane-bound phosphatidylcholine and phosphatidylinositol when it is needed for synthesis of **eicosanoids** (20-carbon fatty acids).

Another phospholipid, phosphatidylinositol, also participates in cell functions. For example, it plays a specific role in anchoring membrane proteins when the proteins are covalently attached to lipids. This function has been demonstrated by the fact that certain membrane proteins are released when cells are treated with a phosphatidylinositol-specific phospholipase C, which hydrolyzes the ester bond connecting the glycerol to the phosphate. Phosphatidylinositols anchor a wide variety of surface antigens and other surface enzymes in eukaryotic cells. In addition, certain hydrolytic products of phosphatidylinositol are active in intracellular signaling and act as second messengers in hormone stimulation. An example of this role is the mechanism of action of insulin (discussed in Chapter 3). Phosphatidylinositol in the plasma membrane can be doubly phosphorylated by ATP, forming phosphatidylinositol-4, 5-bisphosphate. Stimulation of the cell by certain hormones, such as insulin, activates a specific phospholipase C, which produces inositol-1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol-4,5-bisphosphate. Both of these products function as second messengers in cell signaling. Inositol-1,4,5-trisphosphate causes the release of Ca²⁺ held within membrane-bounded compartments of the cell, triggering the activation of a variety of Ca²⁺-dependent enzymes and hormonal responses [2]. Diacylglycerol binds to and activates an enzyme, protein kinase C, which transfers phosphate groups to several cytoplasmic proteins, thereby altering their enzymatic activities [3,4]. This dual-signal hypothesis of phosphatidylinositol hydrolysis is represented in Figure 5.7.

Sphingolipids

The 18-carbon amino alcohol sphingosine forms the backbone of the **sphingolipids**. Sphingosine (Figure 5.8) typically combines with a long-chain fatty acid through an amide linkage to form ceramide. Lipids formed from sphingosine are categorized into three subclasses: sphingomyelins, cerebrosides, and gangliosides. Of these, only the sphingomyelins are sphingophosphatides (Figure 5.9). The other two subclasses of sphingolipids contain no phosphate but instead possess a carbohydrate moiety. They are called glycolipids and are discussed in the next section. Sphingomyelins occur in plasma membranes of animal cells and are found in particularly large amounts in the myelin sheath of nerve tissues. The sphingomyelins contain ceramide (a fatty acid residue attached in an amide linkage to the amino group of the sphingosine), which in turn is esterified to phosphorylcholine (Figure 5.9). Sphingomyelins can combine with either phosphorylcholine or phosphoylethanolamine. Sphingomyelins are important in the nervous system (e.g., in the myelin sheath) in higher animals.

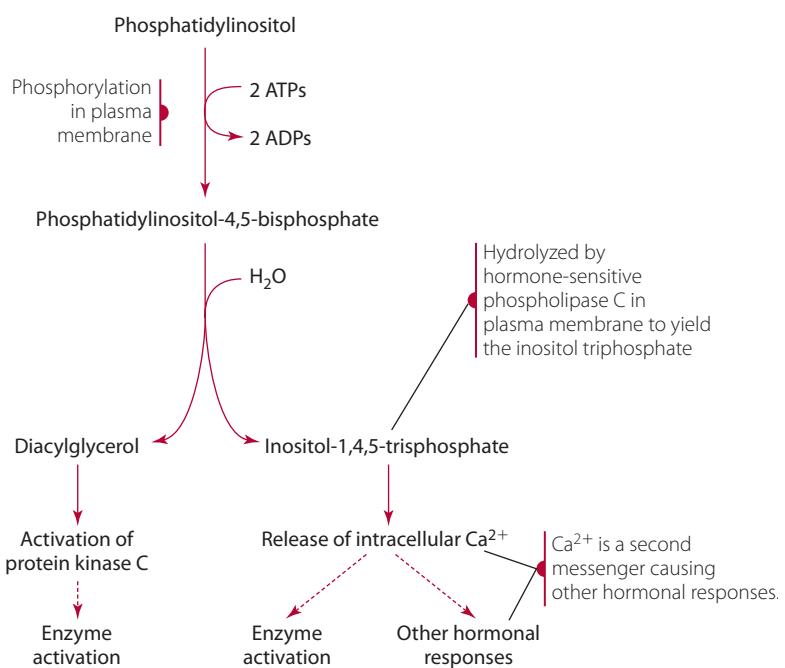


Figure 5.7 Phosphatidylinositol-4,5-bisphosphate, formed in the plasma membrane by phosphorylation of phosphatidylinositol.

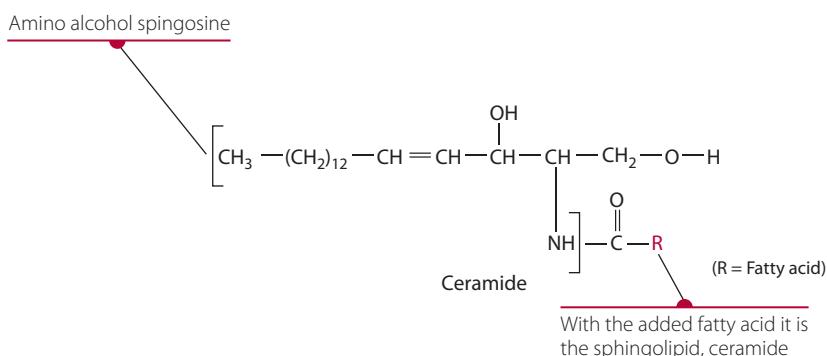


Figure 5.8 Structure of the sphingolipid ceramide.

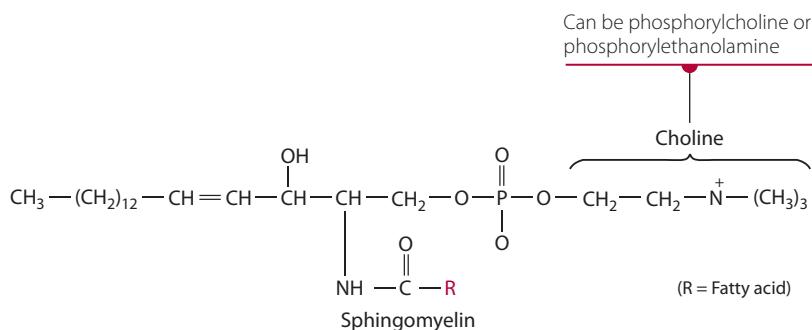


Figure 5.9 The structure of sphingomyelin.

GLYCOLIPIDS

Glycolipids can be subclassified into cerebrosides and gangliosides. They are so named because they have a carbohydrate component within their structure. Like the phospholipids, their physiological role is principally structural, contributing little as an energy source. Cerebrosides and gangliosides occur in the medullary sheaths of nerves and in brain tissue, particularly the white matter. As in the

case of sphingomyelin, the sphingosine moiety provides the backbone for glycolipid structure. It is attached to a fatty acid by an amide bond, forming ceramide, as discussed previously. The glycolipids do not contain phosphate.

A cerebroside is characterized by the linking of ceramide to a monosaccharide unit such as glucose or galactose, producing either a glucocerebroside or a galactocerebroside (Figure 5.10).

Sugar can be glucose (glucocerebroside) or a galactose (galactocebroside).

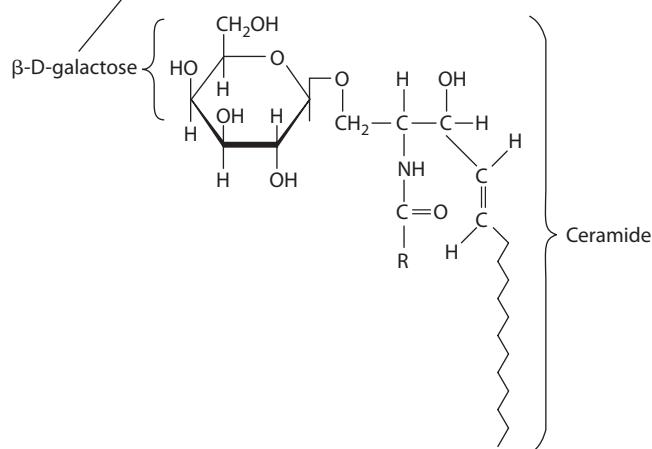


Figure 5.10 A galactocebroside.

Gangliosides resemble cerebrosides, except that the single monosaccharide unit of the cerebroside is replaced by an oligosaccharide containing various monosaccharide derivatives, such as N-acetyl neuraminic acid and N-acetyl galactosamine. Gangliosides are known to be involved in certain recognition events that occur at the cell surface. For example, they provide the carbohydrate determinants of the human blood groups A, B, and O.

Digestion

Because fats are hydrophobic, their digestion poses a special problem in that digestive enzymes, like all proteins, are hydrophilic and normally function in an aqueous environment. The dietary lipid targeted for digestion is emulsified by a very efficient process, mediated mainly by bile salts. This emulsification greatly increases the surface area of the dietary lipid targeted for digestion. Consequently, the accessibility of the fat to digestive enzymes is greatly increased by bile salt action.

Triacylglycerols, phospholipids (primarily phosphatidylcholine), and sterols (mainly cholesterol) provide the lipid component of the typical Western diet. Of these, triacylglycerols, customarily called fats or triglycerides, are by far the major contributor, with a consumption rate of about 150 g daily on average. Compare this to the intake of cholesterol, which is typically 300 to 600 mg per day, depending on the quantity of animal products in the diet. Digestive enzymes involved in breaking down dietary lipids in the gastrointestinal tract are esterases that cleave the ester bonds within triacylglycerols (lipase), phospholipids (phospholipases), and cholesteryl esters (cholesterol esterase).

TRIACYLGLYCEROL DIGESTION

Most dietary triacylglycerol digestion is completed in the lumen of the small intestine, although the process actually begins in the stomach with lingual lipase released by the serous gland, which lies beneath the tongue, and gastric lipase produced by the chief cells of the stomach. Basal secretion of these lipases apparently occurs continuously but can be stimulated by neural (sympathetic agonists), dietary (high fat), and mechanical (sucking and swallowing) factors. These lipases account for much of the limited digestion (10%–30%) of TAG that occurs in the stomach. The lipase activity is made possible by the enzymes' particularly high stability at the low pH of the gastric juices. Gastric lipase readily penetrates milk fat globules without substrate stabilization by bile salts, a feature that makes it particularly important for fat digestion in the suckling infant, whose pancreatic function may not be fully developed. Both lingual and gastric lipases act preferentially on triacylglycerols containing medium- and short-chain fatty acids. They preferentially hydrolyze fatty acids at the *sn*-3 position, releasing a fatty acid and 1,2-diacylglycerols as products. This specificity again is advantageous for the suckling infant because in milk, triacylglycerols' short- and medium-chain fatty acids are usually esterified at the *sn*-3 position. Short- and medium-chain fatty acids are metabolized more directly than are long-chain fatty acids. This structural specificity is also an advantage of the commercially available high-energy formulas for premature infants. These products supply ample energy to the premature infant in a very small volume [5].

For dietary fat in the stomach to be hydrolyzed by lingual and gastric lipases, some degree of emulsification must occur to expose a sufficient surface area of the substrate. Muscle contractions of the stomach and the squirting of the fat through a partially opened pyloric sphincter produce shear forces sufficient for emulsification. Also, potential emulsifiers in the acid milieu of the stomach include complex polysaccharides, phospholipids, and peptic digests of dietary proteins. The presence of undigested lipid in the stomach delays the rate at which the stomach contents empty, presumably by way of the hormone enterogastrone (GIP and secretin; Chapter 2), which inhibits gastric motility. Dietary fats therefore have a “high satiety value.”

Most TAG digestion occurs in the small intestine. Significant hydrolysis and absorption, especially of the long-chain fatty acids, require less acidity, appropriate lipases, more effective emulsifying agents (bile salts), and specialized absorptive cells. These conditions are provided in the lumen of the upper small intestine. The partially hydrolyzed lipid emulsion leaves the stomach and enters the duodenum as fine lipid droplets. Effective emulsification takes place because as mechanical shearing continues, it is complemented by bile that is released from the gallbladder as a result of stimulation by the hormone cholecystokinin (CCK).

Refer to Chapter 2 for a discussion of the role of bile in digestion and the synthesis of bile from cholesterol. Figure 5.5 shows the oxidation of cholesterol to form cholic acid. The formation of conjugated bile salts from cholic acid is shown in Figure 5.11. Bicarbonate is released simultaneously with the release of pancreatic lipase, elevating the pH to a level suitable for pancreatic lipase activity. In combination with triacylglycerol breakdown products, bile salts are excellent emulsifying agents. Their emulsifying effectiveness is due to their **amphipathic** properties, that is, they possess both hydrophilic and hydrophobic “ends.” Such molecules tend to arrange themselves on the surface of small fat particles, with their

hydrophobic ends turned inward and their hydrophilic regions turned outward toward the water phase. This chemical action, together with the help of peristaltic agitation, converts the fat into small droplets with a greatly increased surface area. The particles then can be readily acted on by pancreatic lipase.

The Role of Colipase

Pancreatic lipase activation is complex, requiring the participation of the protein colipase, calcium ions, and bile salts. Colipase is formed by the hydrolytic activation by trypsin of procolipase, also of pancreatic origin. It contains approximately 100 amino acid residues and possesses

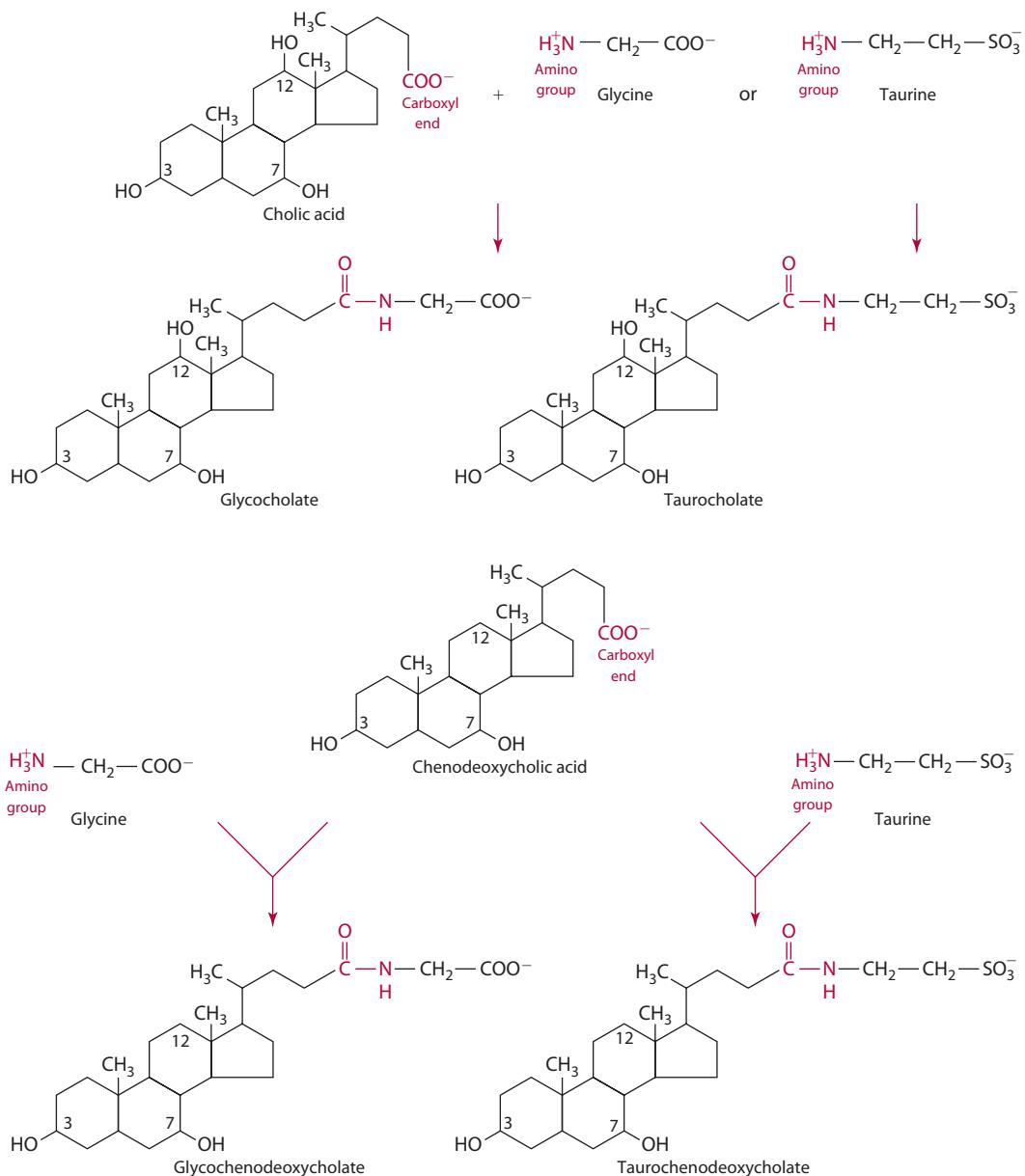


Figure 5.11 The formation of glycocholate, taurocholate, glycochenodeoxycholate, and taurochenodeoxycholate conjugated bile acids.

distinctly hydrophobic regions that are believed to act as lipid-binding sites. Colipase has been shown to associate strongly with pancreatic lipase and therefore may act as an anchor, or linking point, for attachment of the enzyme to the bile salt-stabilized micelles (described in the next section).

The action of pancreatic lipase on ingested triacylglycerols results in a complex mixture of diacylglycerols, monoacylglycerols, and free fatty acids. Its specificity is primarily toward *sn*-1-linked fatty acids and secondarily to *sn*-3 bonds. Therefore, the main path of this digestion progresses from triacylglycerols to 2,3-diacylglycerols to 2-monoacylglycerols. Only a small percentage of the triacylglycerols is hydrolyzed totally to free glycerol. The complete hydrolysis of triacylglycerols that does occur probably follows the isomerization of the 2-monoacylglycerol to 1-monoacylglycerol, which is then hydrolyzed.

CHOLESTEROL AND PHOSPHOLIPID DIGESTION

Cholesterol esters and phospholipids are hydrolyzed by a specific process described here. Esterified cholesterol undergoes hydrolysis to free cholesterol and a fatty acid in a reaction catalyzed by the enzyme cholesterol esterase.

The C-2 fatty acid of lecithin is hydrolytically removed by a specific esterase, phospholipase A₂, producing lysolecithin and a free fatty acid. The products of the partial digestion of lipids, primarily 2-monoacylglycerols, lysolecithin, cholesterol, and fatty acids, combine with bile salts to form negatively charged polymolecular aggregates called micelles. These aggregates have a much smaller diameter (~5 nm) than the unhydrolyzed precursor particles, allowing them access to the intramicrovillus spaces (50–100 nm) of the intestinal membrane. A summary of the digestion of lipids is shown in Table 5.3 and Figure 5.12.

Table 5.3 Overview of Triacylglyceride Digestion

Location	Major Events	Required Enzyme or Secretion	Details
Mouth	Triglyceride ↓ Minor amount of digestion Triglycerides, diglycerides, and fatty acids	Lingual lipase produced in the salivary glands	<p>Diglyceride</p>
Stomach	Additional digestion ↓ Triglycerides, diglycerides, and fatty acids	Gastric lipase produced in the stomach	<p>Diglyceride</p>
Small intestine	Phase I: Emulsification ↓ Emulsified triglycerides, diglycerides, and fatty acid micelles Phase II: Enzymatic digestion ↓ Monoglycerides and fatty acids	Bile; no lipase Pancreatic lipase produced in pancreas	<p>Monoglyceride</p>

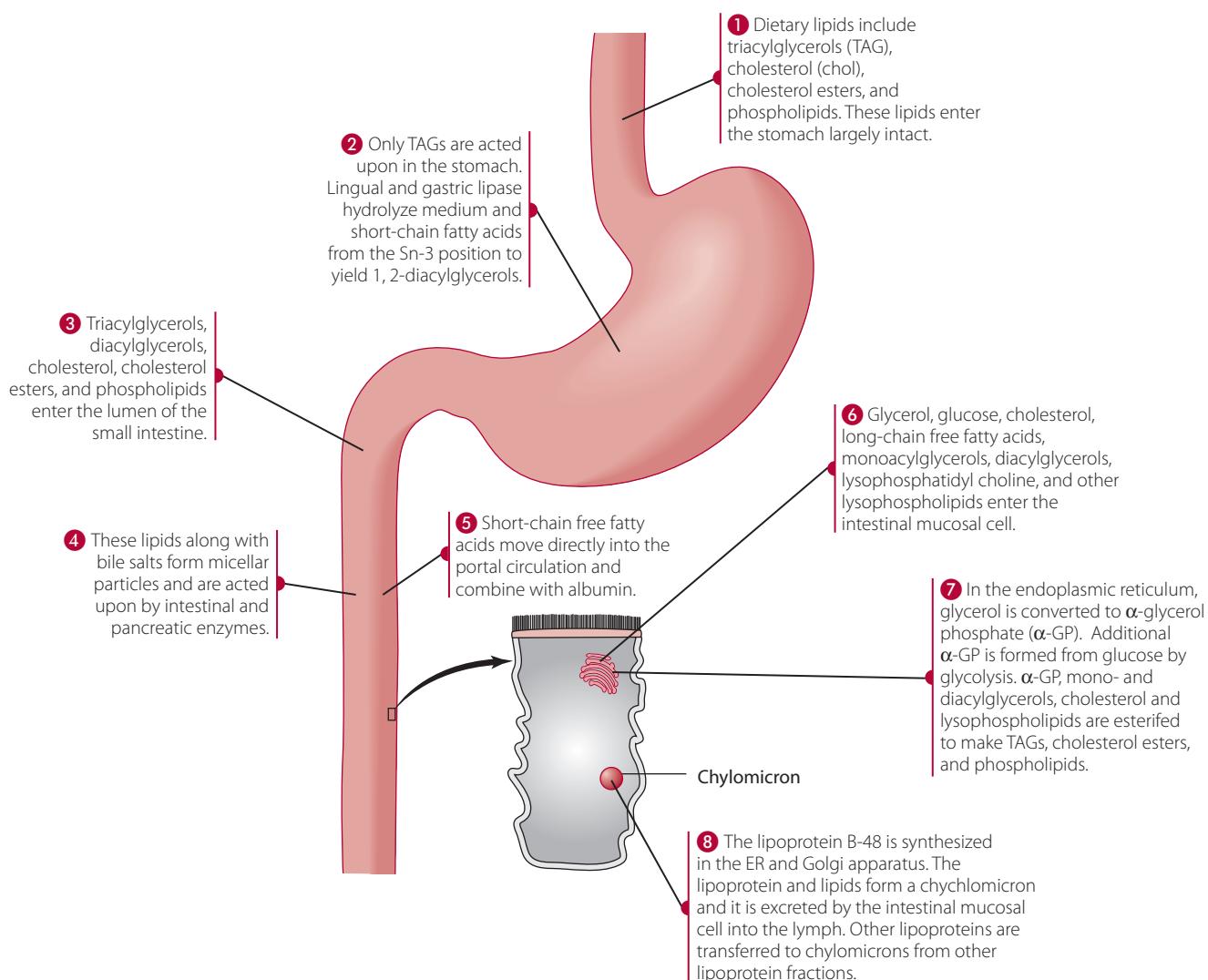


Figure 5.12 Summary of digestion and absorption of dietary lipids.

Absorption

Stabilized by the polar bile salts, the micellar particles are sufficiently water soluble to penetrate the unstirred water layer that bathes the absorptive cells of the small intestine. The absorptive cells are called intestinal mucosal cells, or enterocytes. Micelles interact at the brush border of these cells, whereupon the lipid contents of the micelles (which include FFA, 2-monoacylglycerols, cholesterol, cholesterol esters, and lysolecithin) diffuse out of the micelles and into the enterocytes, moving down a concentration gradient. Although this process occurs in the distal duodenum and the jejunum, the bile salts are not absorbed at this point but instead are absorbed in the ileal segment of the small intestine. From there the bile salts are returned to the liver by way of the portal vein to be resecreted in the bile. This circuit is called enterohepatic circulation of the bile salts (see Chapter 2).

After the absorption of free fatty acids, 2-monoacylglycerols, cholesterol, and lysophosphatidylcholine into the enterocytes, intracellular re-formation of triacylglycerols, phosphatidylcholine, and cholesteryl esters takes place. However, the process is a function of the chain length of the fatty acids involved. Fatty acids that have more than 10 to 12 carbon atoms are first activated by being coupled to coenzyme A by the enzyme acyl CoA synthetase. They are then re-esterified into triacylglycerols, phosphatidylcholine, and cholesteryl esters, as mentioned earlier. Short-chain fatty acids (those containing fewer than 10 to 12 carbon atoms), in contrast, pass from the cell directly into the portal blood, where they bind with albumin and are transported directly to the liver. The different fates of the long- and short-chain fatty acids result from the specificity of the acyl CoA synthetase enzyme for long-chain fatty acids only. Key features of intestinal absorption of lipid digestion products are depicted in Figure 5.12.

Note that triacylglycerols can also be synthesized from α -glycerophosphate in the enterocytes. This metabolite can be formed either from the phosphorylation of free glycerol or from reduction of dihydroxyacetone phosphate, an intermediate in the pathway of glycolysis (Figure 3.17). Triacylglycerol synthesis by this route is also shown in Figure 5.12.

Transport and Storage

LIPOPROTEINS

Lipids resynthesized in the enterocytes, together with fat-soluble vitamins, are collected in the cell's endoplasmic reticulum as large fat particles. While still in the endoplasmic reticulum, the particles receive a layer of lipoprotein B-48 on their surface. This lipoprotein is an abridged version of the lipoprotein produced by the liver. It stabilizes the particles in the aqueous environment of the circulation, which they eventually enter. The particles are pinched off as lipid vesicles, which then fuse with the Golgi apparatus. There, carbohydrate is attached to the protein coat, and the completed particles, called chylomicrons, are transported to the cell membrane and exocytosed into the lymphatic circulation. While chylomicrons are in circulation, additional lipoproteins are transferred to them from other lipoprotein particles. Chylomicrons belong to a family of compounds called **lipoproteins**, which get their name from the fact that they are made of lipids and proteins.

Lipoproteins play an important role in transporting lipids, and serum lipoprotein patterns have been implicated as risk factors in chronic cardiovascular disease.

The protein portion of any lipoprotein is called the **apolipoprotein**. Apolipoproteins play a very important role in the structural and functional relationship among the lipoproteins. Each of the lipoprotein particles contains one or more apoprotein. In general, each lipoprotein particle has only a single molecule of each apolipoprotein found in the particle. This fact allows the blood levels of these apoproteins to be used to evaluate potential cardiovascular disease risk (CVD). CVD risk will be discussed further in Chapter 7.

Chylomicrons transport exogenous dietary lipids. Lipoproteins other than chylomicrons transport endogenous lipids, which are circulating lipids that do not arise directly from intestinal absorption but instead are processed through other tissues, such as the liver. Several types of lipoproteins exist, differing in their lipid composition, apolipoprotein composition, physical properties, and metabolic function. Initially, lipoproteins were separated from serum by electrophoresis and therefore were named based on their movement in an electrical gradient. Later, they were separated by centrifugation and were named based on their density. These names persist even though other methods are often used for their separation. Lipoproteins with higher concentrations of lipid have a lower density. Very low density lipoproteins (VLDLs or pre- β -lipoprotein) are made in the liver; the primary function of these lipoproteins is to transport triacylglycerol made by the liver to other, non-hepatic tissues. VLDLs also contain cholesterol and cholesteryl esters.

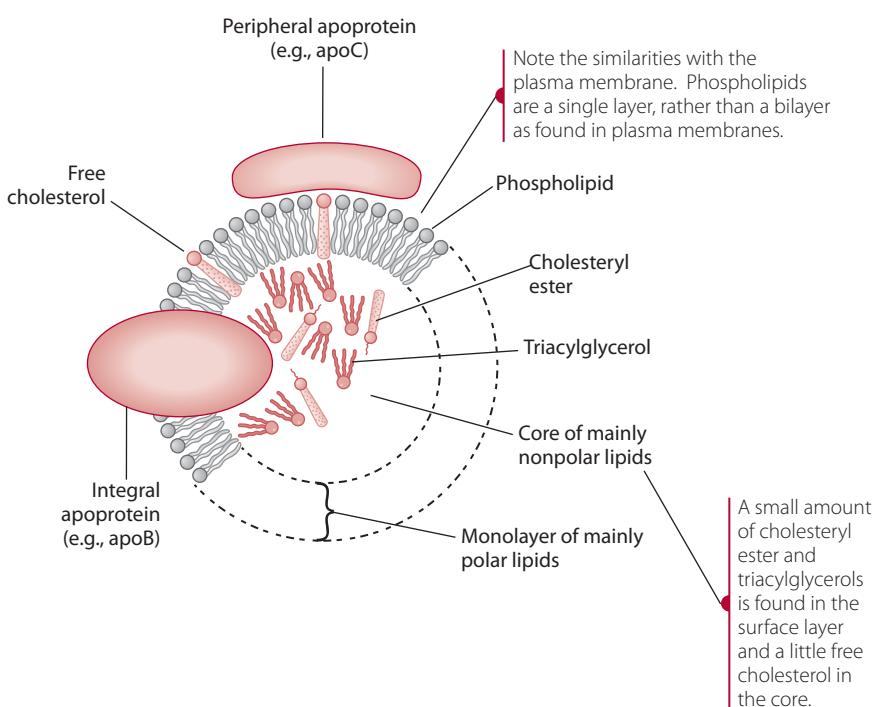


Figure 5.13 Generalized structure of a plasma lipoprotein.

As TAG is removed from these lipoproteins, they undergo a brief stage as intermediary lipoprotein (IDL). As further TAG is removed, IDLs become low-density lipoproteins (LDL). When LDLs were separated by electrophoresis, they were called β -lipoproteins.

The role that all lipoproteins share is transporting lipids from tissue to tissue to supply the lipid needs of different cells. The arrangement of the lipid and protein components of a typical lipoprotein particle is represented in Figure 5.13. Note that more hydrophobic lipids (such as TAG and cholestryl esters) are located in the core of the particle, whereas the relatively more polar proteins and phospholipids are situated on the surface. This structure enhances their stability in an aqueous environment. As previously stated, lipoproteins differ according to the ratio of lipid to protein within the particle as well as in having different proportions of lipid types: triacylglycerols, cholesterol and cholestryl esters, and phospholipids. Such compositional differences

influence the density of the particle, which has become the physical characteristic used to differentiate and classify the various lipoproteins. In order of lowest (the most lipid) to highest density, the lipoprotein fractions are chylomicrons, very low density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs). An intermediate-density particle (IDL) also exists, which has a density between that of VLDL and LDL. The IDL particles are very short lived in the bloodstream, however, and have little nutritional or physiological importance. Figure 5.14 shows the lipid and protein makeup of each of the lipoproteins.

Apolipoproteins

Apolipoproteins, the protein components of lipoproteins, tend to stabilize the lipoproteins as they circulate in the aqueous environment of the blood, but they also have other important functions. They confer specificity on the

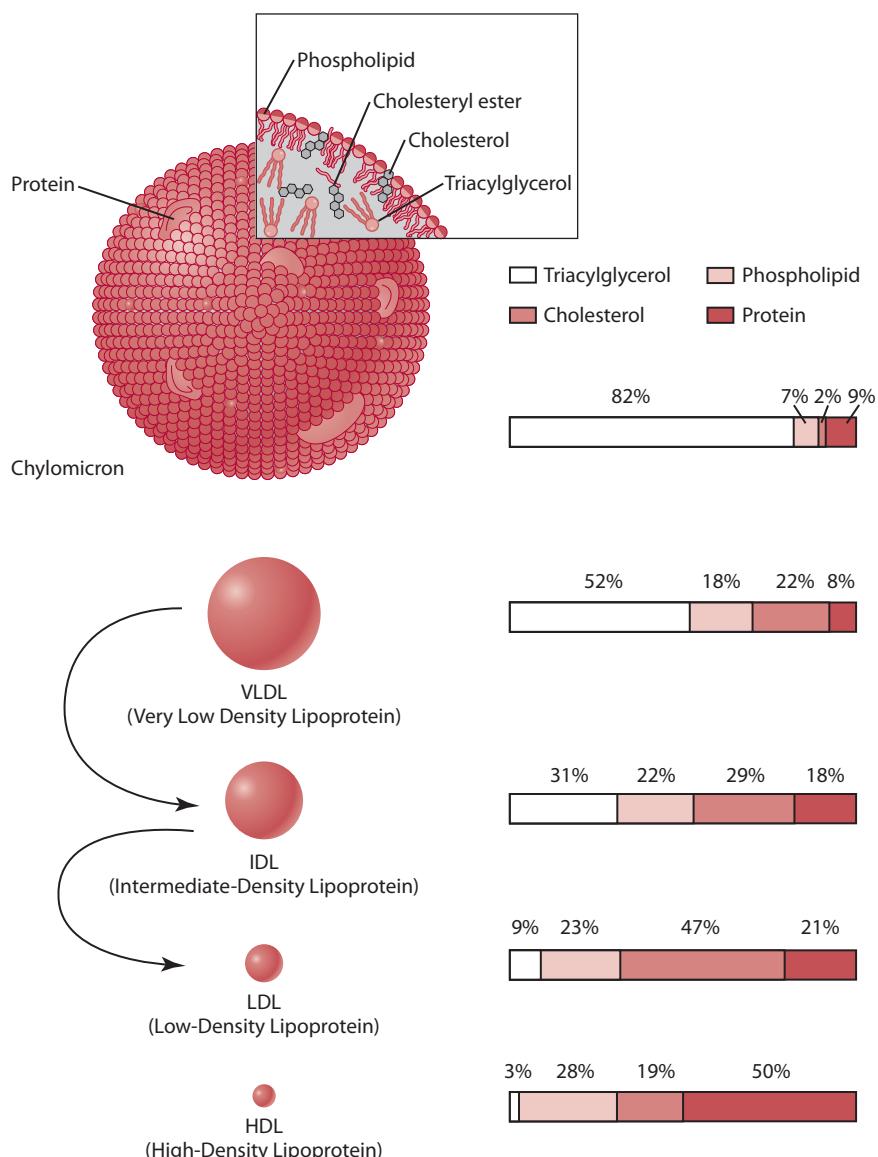


Figure 5.14 Lipid and protein of lipoprotein fractions.

lipoprotein complexes, allowing them to be recognized by specific receptors on cell surfaces. Apolipoproteins also stimulate certain enzymatic reactions, which in turn regulate the lipoproteins' metabolic functions.

A series of letters (A to E), with subclasses of each, are used to identify the various apolipoproteins. For convenience, they are usually abbreviated "apo" and followed by the identifying letter—that is, apoA-1, apoB-100, apoC-2, and so on. A partial listing of the apolipoproteins, together with their molecular weight, the lipoprotein with which they are associated, and their postulated physiological function, is found in Table 5.4. A brief overview of the lipoprotein function is provided here. A more detailed description of their metabolism follows.

Chylomicrons

As discussed above, re-formed lipid derived from exogenous sources leaves the enterocytes (intestinal mucosal cells) largely in the form of chylomicrons, though some HDL is produced by the enterocytes as well. **Chylomicrons** are the primary form of lipoprotein formed from exogenous (dietary) lipids. The role of the chylomicron is to deliver dietary lipid mostly to tissues other than the liver, such as muscle and adipose tissue (80%). Much of the lipid delivered to the liver is in the form of chylomicron remnants (20%). Triacylglycerols are the most abundant lipid in the diet, and are also the most abundant lipid in chylomicrons. As lipids are removed from chylomicrons, they undergo intravascular conversion to chylomicron remnants (structurally similar to VLDL). Chylomicrons first appear in the lymphatic vessels of the abdominal region and then enter

the bloodstream at a slow rate, which prevents large-scale changes in the lipid content of peripheral blood. Entry of chylomicrons into the blood from the lymph can continue for up to 14 hours after consumption of a large meal rich in fat. The peak level of lipid in blood plasma usually occurs 30 minutes to 3 hours after a meal and returns to near normal within 5 to 6 hours. This time can vary, however, depending on the stomach emptying time, which in turn depends on the size and composition of the meal.

Chylomicrons are transported by the blood throughout all tissues in the body, while undergoing intravascular hydrolysis at certain tissue sites. This hydrolysis occurs through the action of the enzyme lipoprotein lipase, which is associated with the endothelial cell surface of the small blood vessels and capillaries within non-hepatic tissue such as adipose and muscle. Its extracellular action on the circulating particles releases free fatty acids and diacylglycerols, which are quickly absorbed by the tissue cells. The blood vessels of the liver do not contain this lipoprotein lipase. The large, triacylglycerol-laden chylomicrons account for the turbidity (milky appearance) of postprandial plasma. Because lipoprotein lipase is the enzyme that solubilizes these particles by its lipolytic action, it is sometimes referred to as "clearing factor." The part of the chylomicron that is left following this lipolytic action is called a **chylomicron remnant**—a smaller particle, relatively less rich in triacylglycerol, but richer in cholesterol and cholesteryl esters. These remnants are removed from the bloodstream by liver cell endocytosis following interaction of the remnant particles with specific receptors for apolipoprotein E or B/E on the cells [6].

Table 5.4 Apolipoproteins of Human Plasma Lipoproteins

Apolipoprotein	Lipoprotein	Molecular Mass (Da)	Additional Remarks
apoA-1	HDL, chylomicrons	28,000	Activator of lecithin: cholesterol acyltransferase (LCAT). Ligand for HDL receptor.
apoA-2	HDL, chylomicrons	17,000	Structure is two identical monomers joined by a disulfide bridge. Inhibitor of LCAT?
apoA-4	Secreted with chylomicrons but transfers to HDL	46,000	Associated with the formation of triacylglycerol-rich lipoproteins. Function unknown.
apoB-100	LDL, VLDL, IDL	550,000	Synthesized in liver. Ligand for LDL receptor.
apoB-48	Chylomicrons, chylomicron remnants	260,000	Synthesized in intestine.
apoC-1	VLDL, HDL, chylomicrons	7,600	Possible activator of LCAT.
apoC-2	VLDL, HDL, chylomicrons	8,916	Activator of extrahepatic lipoprotein lipase.
apoC-3	VLDL, HDL, chylomicrons	8,750	Several polymorphic forms depending on content of sialic acids.
apoD	Subfraction of HDL	20,000	Function unknown.
apoE	VLDL, HDL, chylomicrons, chylomicron remnants	34,000	Present in excess in the β -VLDL of patients with type III hyperlipoproteinemia. The sole apoprotein found in HDL _c of diet-induced hypercholesterolemic animals. Ligand for chylomicron remnant receptor in liver and LDL receptor.

Very Low Density Lipoprotein (VLDL) and Low-Density Lipoproteins (LDL)

Very low density lipoproteins are produced in the liver from endogenous triacylglycerol in much the same way as chylomicrons were produced in the enterocytes. The lipid is synthesized in the smooth ER, transferred to the Golgi apparatus, and excreted from the cell along with the apolipoproteins B-100, apoC, and apoE. Nascent VLDL of liver origin is stripped of triacylglycerol by lipoprotein lipase at extracellular sites, resulting in the formation of a transient IDL particle and, finally, a cholesterol-rich LDL. The apolipoprotein apoC-2, an activator of lipoprotein lipase, is a component of both chylomicrons and VLDL, as indicated in Table 5.4. These particles are subject to lipoprotein lipase action and are an example of the regulatory function of an apolipoprotein. Within the muscle cell, the free fatty acids from VLDL and those derived from hydrolysis of the absorbed diacylglycerols are primarily oxidized for

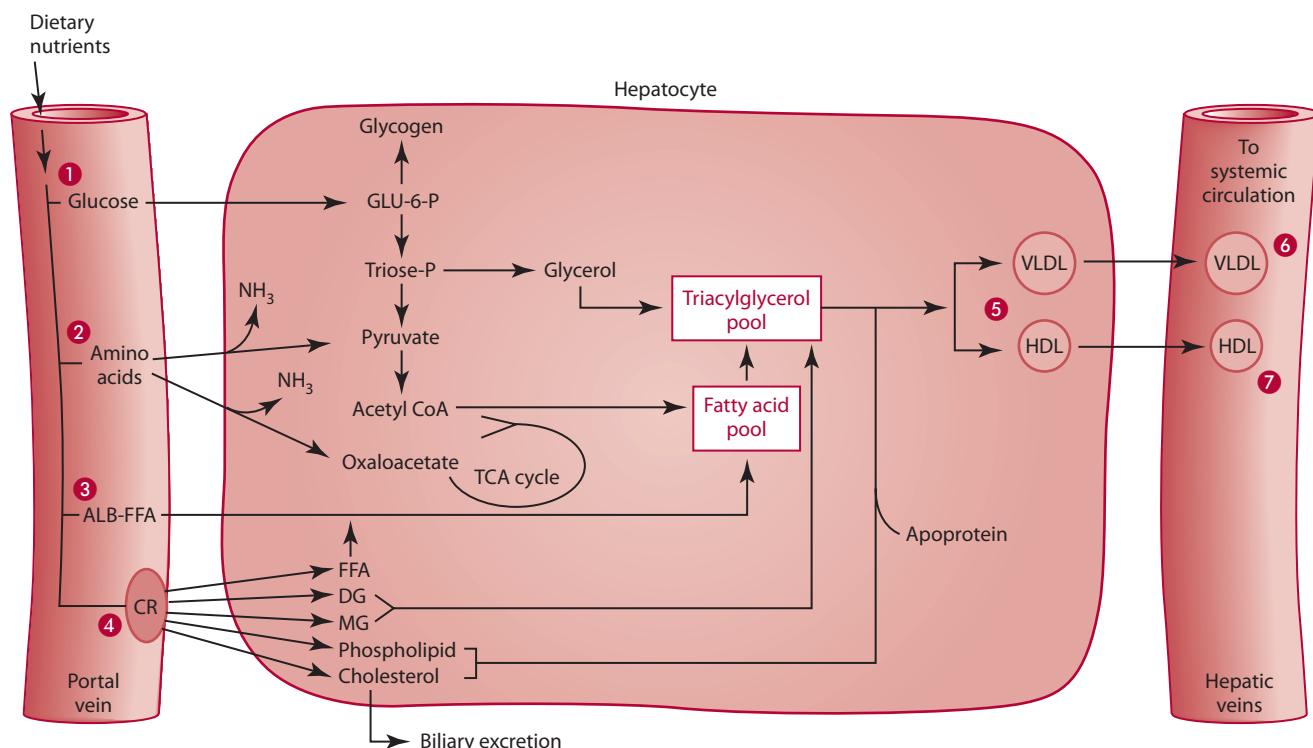
energy, with only limited amounts resynthesized for storage as triacylglycerols. Endurance-trained muscle, however, does contain triacylglycerol deposits.

In adipose tissue, in contrast, the absorbed fatty acids are largely used to synthesize triacylglycerols, in keeping with that tissue's storage role. In this manner, chylomicrons and VLDL are cleared from the plasma in a matter of minutes and a few hours, respectively, from the time they enter the bloodstream. Figure 5.15 summarizes lipid metabolism in a hepatocyte following a fatty meal.

ROLE OF THE LIVER AND ADIPOSE TISSUE IN LIPID METABOLISM

This section explains how the liver and adipose tissues are involved in lipid metabolism following a meal.

Our normal eating pattern is to consume a meal, followed by several hours of fasting before we eat again. Our bodies



- ① Dietary nutrients enter the liver through the portal vein. Glucose can be converted to glycogen or enter glycolysis.
- ② Amino acids enter the amino acid pool and some are metabolized to produce pyruvate and oxaloacetate.
- ③ Short-chain free fatty acids (FFA), bound to albumin enter the fatty acid pool and are incorporated into triacylglycerols (TAG).
- ④ Chylomron remnants (CR) attach to binding sites with lipoprotein lipase and deliver FFA, diglycerides (DG), monoglycerides (MG), phospholipids (PL), and cholesterol (C).

- ⑤ TAG, C, and PL are packaged with apolipoproteins and enter the circulation as VLDL or HDL.
- ⑥ VLDL deliver the meal's lipids to the non-hepatic tissue.
- ⑦ HDL is involved in reverse cholesterol transport.

Figure 5.15 Metabolism in the liver following a fatty meal.

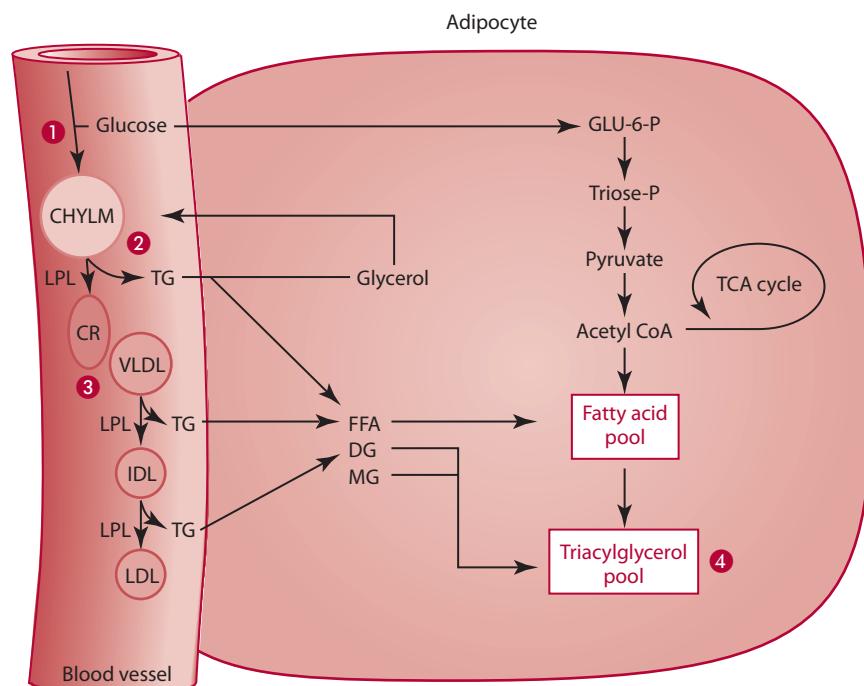
have adapted to cope with the time of extra nutrients in the blood followed by a period in which the levels of blood nutrients must be restored from tissue storage. A brief discussion of this process is provided in this section. A more in-depth description of fasting's effects on metabolism is presented in Chapter 7, which covers the interrelationships in the metabolism of energy-yielding nutrients.

Liver

The liver plays a very important role in the body's use of lipids and lipoproteins. As discussed earlier, hepatic synthesis of the bile salts, indispensable for digesting and absorbing dietary lipids, is one of its functions. In addition, the liver is the key player in lipid transport, because it is the site of synthesis of lipoproteins formed from endogenous lipids and apoproteins. The liver is capable of synthesizing new lipids from nonlipid precursors, such as glucose and amino acids. It can also take up and catabolize exogenous lipids delivered to it in the form of chylomicron remnants, repackaging their lipids into HDL and VLDL forms. Remember that pathways of lipid, carbohydrate, and protein metabolism are integrated and cannot stand alone. Figure 5.16 summarizes exogenous lipid metabolism following a fatty meal.

In the postprandial (fed) state, glucose, amino acid, and short-chain fatty acid concentrations rise in portal blood, which goes directly to the liver. In the hepatocyte, glucose is phosphorylated for use, and glycogen subsequently is synthesized until the hepatic glycogen stores are repleted. If portal hyperglycemia persists (more glucose comes from the digestive system), glucose is converted to fatty acids. Remember from Chapter 3 that glucose is metabolized by glycolysis to triose phosphates, to pyruvate, and then to acetyl CoA. The acetyl CoA is used to synthesize fatty acids and the glycerol is made from triose phosphates (such as glycerol 3-phosphate). Amino acids can also serve as precursors for lipid synthesis, because they can be metabolically converted to acetyl CoA and pyruvate. The synthesis of fatty acids, triacylglycerols, and glycerophosphatides is described in detail later in this chapter.

In addition to the newly synthesized lipid derived from nonlipid precursors, there is also the exogenous lipid delivered to the liver, derived from chylomicron remnants and short-chain fatty acids that were excreted from the intestine directly into the portal blood. The apolipoprotein E on the surface of the chylomicron remnants binds with specific receptors for apoE in the vascular



- 1 Glucose is metabolized to make acetyl CoA which can be converted to fatty acids.
- 2 Lipoprotein lipase act on TAG in chylomicrons (CHYLM) and free fatty acids (FFA) and glycerol enters the adipocyte. Glycerol can not be used and is excreted back into the bloodstream.
- 3 Lipoprotein lipase acts on VLDL so TAG, FFA, diglycerides (DG), monoglycerides (MG) and cholesterol enter the cell.
- 4 The pathways favor energy storage as TAG. Insulin stimulates lipogenesis by promoting glucose into the cell and by inhibiting the lipase which hydrolyzes the stored TAG to FFA and glycerol.

Figure 5.16 Lipid metabolism in the adipose cell following a meal.

endothelial cells of the liver. The lipid portion of the chylomicron remnant is hydrolyzed and absorbed into the hepatocyte as free fatty acids, monoacylglycerols, diacylglycerols, glycerol, and cholesterol. Resynthesis of these compounds promptly occurs in a manner analogous to the events in the intestinal mucosal cell. An alternate fate of the lipid entering the hepatocyte is for the fatty acids to be used for energy.

Exogenous free fatty acids of short-chain length delivered directly to the hepatic tissue can be used for energy or, following chain elongation, to resynthesize other lipid fractions. Chylomicron remnant cholesterol and cholesteryl esters may be used in several ways:

- converted to bile salts and secreted in the bile
- secreted into the bile as neutral sterol (such as cholesterol or cholesteryl ester)
- incorporated into VLDL or HDL and released into the plasma

Newly synthesized triacylglycerol is combined with phospholipid, cholesterol, and proteins to form VLDL and HDL, which are released into the circulation. Because triacylglycerols can be formed from glucose, hepatic triacylglycerol production is accelerated when the diet is rich in carbohydrate. The additional triacylglycerols results in VLDL overproduction and may account for the occasional transient hypertriacylglycerolemia in normal people when they consume diets rich in simple sugars. Figure 5.15 summarizes exogenous lipid metabolism following a fatty meal.

The HDL shown in Figure 5.16 are involved in reverse cholesterol transport and, when synthesized in the liver, are smaller than the VLDL and contain less triacylglycerol. HDL also possesses phospholipids and cholesterol in addition to TAG as its major lipid constituents. The role of HDL will be discussed later in this chapter.

Adipose Tissue

Adipose tissue shares with the liver an extremely important role in metabolism lipoproteins. Unlike the liver, adipose is not involved in the uptake of chylomicron remnants or the synthesis of endogenous lipoproteins. Adipose is involved in absorbing TAG and cholesterol from chylomicrons through the action of lipoprotein lipase. Adipocytes are the major storage site for triacylglycerol. TAG is in a continuous state of turnover in adipocytes that is constant lipolysis (hydrolysis), countered by constant re-esterification to form TAG. These two processes are not simply forward and reverse directions of the same reactions but are different pathways involving different enzymes and substrates. Each of the processes is regulated separately by nutritional, metabolic, and hormonal factors, the net effect of which determines the level of circulating fatty acids and the extent of adiposity. A single large globule of fat constitutes over 85% by volume of the adipose cell.

A summary of lipid metabolism in an adipocyte is presented in Figure 5.16. In the fed state, metabolic pathways in adipocytes favor triacylglycerol synthesis. As in the liver, adipocyte triacylglycerol can be synthesized from glucose, a process strongly influenced by insulin. Insulin accelerates the entry of glucose into the adipose cells (the liver does not respond to this action of insulin). Insulin also increases the availability and uptake of fatty acids in adipocytes by stimulating lipoprotein lipase. The glycolytic breakdown of cellular glucose provides a source of glycerophosphate for re-esterification with the fatty acids to form triacylglycerols. Absorbed monoacylglycerols and diacylglycerols also furnish the glycerol building block for this resynthesis. Free glycerol is not used in the adipocyte, which does not contain the enzyme glycerol 3-phosphotase. Plasma glycerol levels have been used as an indication of TAG turnover in adipose tissue. Insulin exerts its lipogenic action on adipose further by inhibiting intracellular lipase, which hydrolyzes stored triacylglycerols, thus favoring TAG accumulation. Intracellular lipase is insulin sensitive, distinguishing it from the intravascular lipoprotein lipase that functions extracellularly.

Metabolism of Triacylglycerol during Fasting

To this point, this section has dealt with the role of the liver and adipose tissue in the fed state. In the fasting state, the metabolic scheme in these tissues shifts. For example, as blood glucose levels diminish, insulin concentration falls, accelerating lipolytic activity in adipose tissue. The lipolytic activity produces free fatty acids and glycerol. Free fatty acids derived from adipose tissue circulate in the plasma in association with albumin and are taken up by the liver or muscle cells and oxidized for energy by way of acetyl CoA formation. In the liver, some of the acetyl CoA is diverted to produce ketone bodies, which can serve as important energy sources for muscle tissue and the brain during fasting and starvation. The liver continues synthesizing VLDL and HDL and releases them into circulation, though these processes are diminished in a fasting situation. Glucose derived from liver glycogen and free fatty acids (transported to the liver from adipose tissue) become the major precursors for the synthesis of endogenous VLDL triacylglycerol. As described previously, this lipoprotein then undergoes catabolism to IDL, transiently, and to LDL by lipoprotein lipase. Most of the plasma HDL is endogenous and is composed mostly of phospholipid and cholesterol, along with apoproteins (chiefly of the apoA series).

METABOLISM OF LIPOPROTEINS

Chylomicrons and chylomicron remnants normally are not present in the blood serum during the fasting state. In the section on chylomicrons you learned that chylomicrons are released from the intestinal endothelial cell (nascent

chylomicron) and contain predominately TAG. They also contain apolipoprotein B-48, which has a molecular weight of 48 kilodaltons. The apoB-48 is a subset of the amino acid sequence of the apoB-100 produced by the liver. The next section discusses the binding sites for lipid transport into the cells. The metabolic fate of chylomicrons is shown in Figure 5.17.

Low-Density Lipoprotein (LDL)

The fasting serum concentration of VLDL is quite low, compared with its concentration in postprandial serum, because of VLDL's rapid conversion to IDL and LDL. Therefore, the major lipoproteins in fasting serum are LDL (derived from VLDL), HDL (synthesized mainly in the liver), and a very small amount of VLDL. As discussed earlier and summarized in Table 5.4, the apolipoproteins may regulate metabolic reactions within the lipoprotein particles and determine to a great extent how the particles interact with each other and with receptors on specific cells.

The LDL fraction is the major carrier of cholesterol, binding about 60% of the total serum cholesterol (Figure 5.14). Its function is to transport the cholesterol to tissues, where it may be used for membrane construction or for conversion into other metabolites, such as the steroid hormones. LDL interacts with LDL B-100 receptors on cells, an event that culminates in the removal of the lipoprotein from the circulation. LDL B-100 receptors are located on liver cells

and on cells of tissues peripheral to the liver, but the liver does not effectively remove the LDL from circulation. The distribution of LDL among tissues may depend on its rate of transcapillary transport as well as on the activity of the LDL receptors on cell surfaces. Once bound to the receptor, the receptor and the LDL particle, complete with its lipid cargo, are internalized together by the cell. The particle's component parts are then degraded by lysosomal enzymes in the cell (Figure 5.18). The next section examines the LDL receptor in greater detail. The discovery of this receptor in the late 1970s and early 1980s was an important biochemical event.

The LDL Receptor: Structure and Genetic Aberrations

The discovery of the LDL receptor is credited to Michael S. Brown, M.D., and Joseph L. Goldstein, M.D., who received the 1985 Nobel Prize in Physiology and Medicine. The discovery stemmed from their seeking the molecular basis for the clinical manifestation of hypercholesterolemia, and their research revealed the following facts about LDL and its connection to cholesterol metabolism.

LDL binds to normal fibroblasts (and other cells, particularly the hepatocytes and cells of the adrenal gland and ovarian corpus luteum) with high affinity and specificity. In mutant cells, however, the binding is very inefficient. Although deficient binding of LDL is characteristic

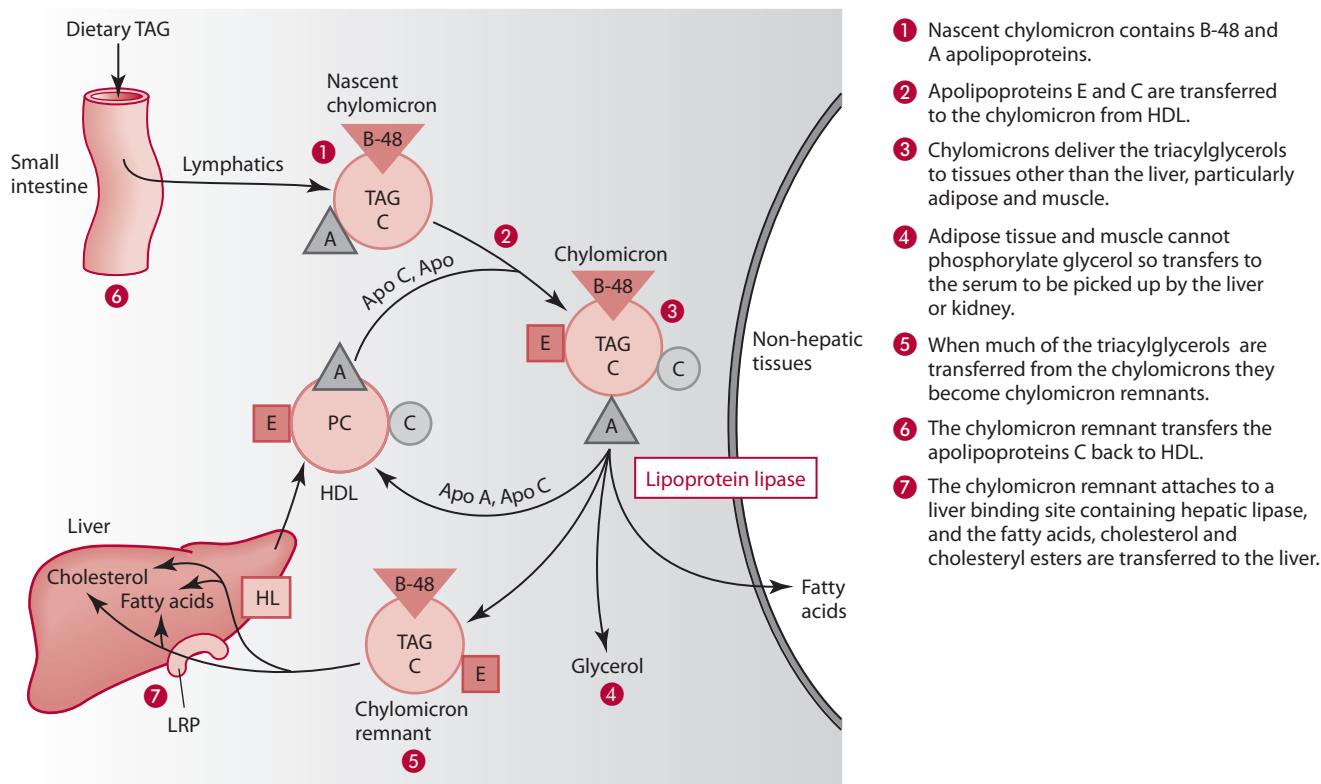


Figure 5.17 Fate of chylomicrons.

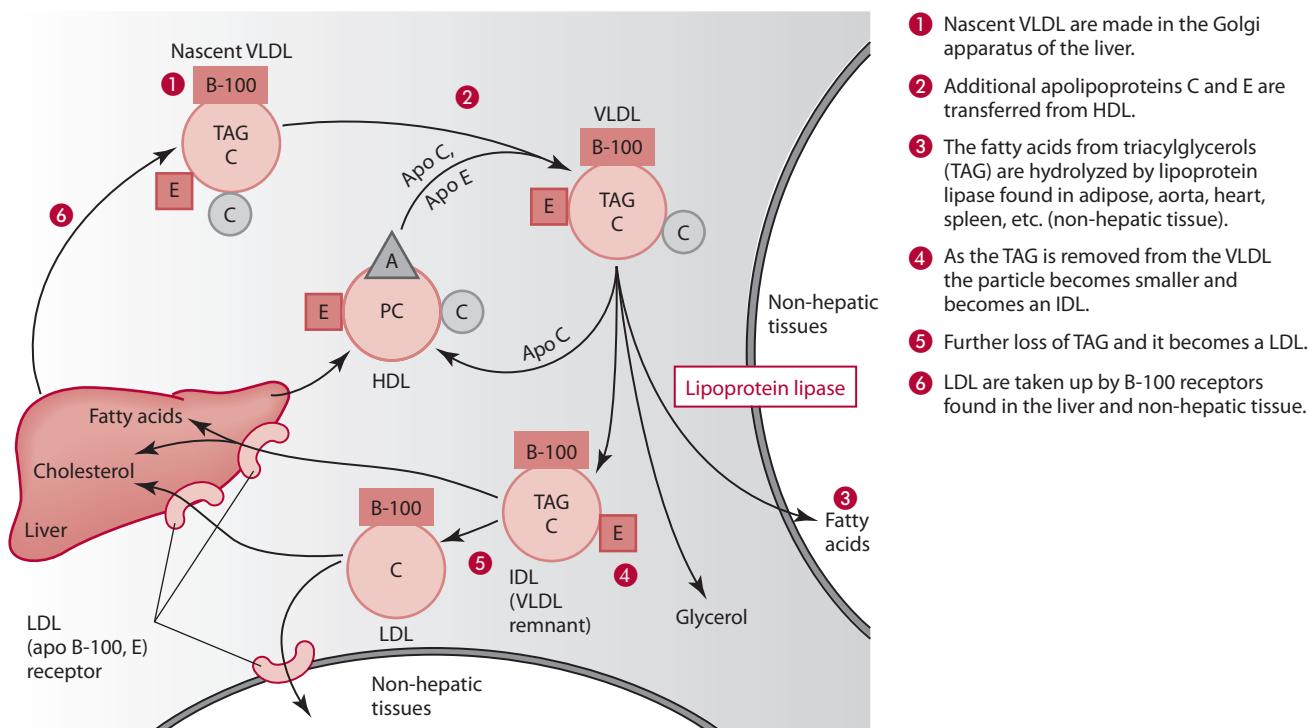


Figure 5.18 Fate of very low density lipoprotein (VLDL) and low-density lipoprotein (LDL).

of all mutant cells, much variation in binding ability exists among different patients with familial homozygous hypercholesterolemia.

Membrane-bound LDL is internalized by endocytosis made possible by receptors that cluster in the LDL-APO B-100 E receptors. Figure 5.19 depicts the fate of the LDL particle following its binding to the membrane receptor. The receptor, having released its LDL, returns to the surface of the cell, making a round trip into and out of the cell every 10 minutes during its 20-hour life span [7]. The dissociated LDL moves into the lysosome, where its protein and cholestryler ester components are hydrolyzed by lysosomal enzymes into amino acids, FFAs, and free cholesterol. The resulting free cholesterol exerts the following regulatory functions:

- It modulates the activity of two microsomal enzymes, 3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA reductase) and acyl CoA: cholestryler acyl transferase (ACAT).
- By lowering the concentration of receptor mRNA, it suppresses synthesis of LDL receptors, thereby preventing further entry of LDL into the cell.

Activity of the HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis, is suppressed through decreased transcription of the reductase gene and the concomitant increased degradation of the enzyme. In contrast, ACAT is activated, promoting formation of cholestryler esters that can be stored as droplets in the cytoplasm of the cell.

Mutant cells unable to bind or internalize LDL efficiently, and thus deprived of the cholesterol needed for membrane synthesis, must obtain the needed cholesterol by synthesizing it. In these cells, HMG CoA reductase is activated, while ACAT is depressed.

LDL receptors interact with apoB-100, the protein carried on the surface of the LDL. The interaction between the receptors and the apoB-100 is the key to the cell's internalization of the LDL. The number of receptors synthesized by cells varies according to cholesterol requirements.

The LDL receptor has been found to be a transmembrane glycoprotein that, in the course of its synthetic process, undergoes several carbohydrate-processing reactions. The carbohydrate moiety is important for proper functioning of the receptor, and its location on the molecule has been mapped. Five domains of the LDL receptor have been identified:

- Domain 1, which is furthest from the membrane and contains the NH₂ terminal of the receptor protein, is rich in cysteine residues. These residues allow the formation of many disulfide bonds that give stability to the molecule. Many of the other amino acid residues in this cysteine-rich domain have negatively charged side chains. This first domain, then, could be the binding site for apoB-100, with its positively charged lysine and arginine residues. These positively charged residues of apoB-100 are known to be crucial for receptor binding.

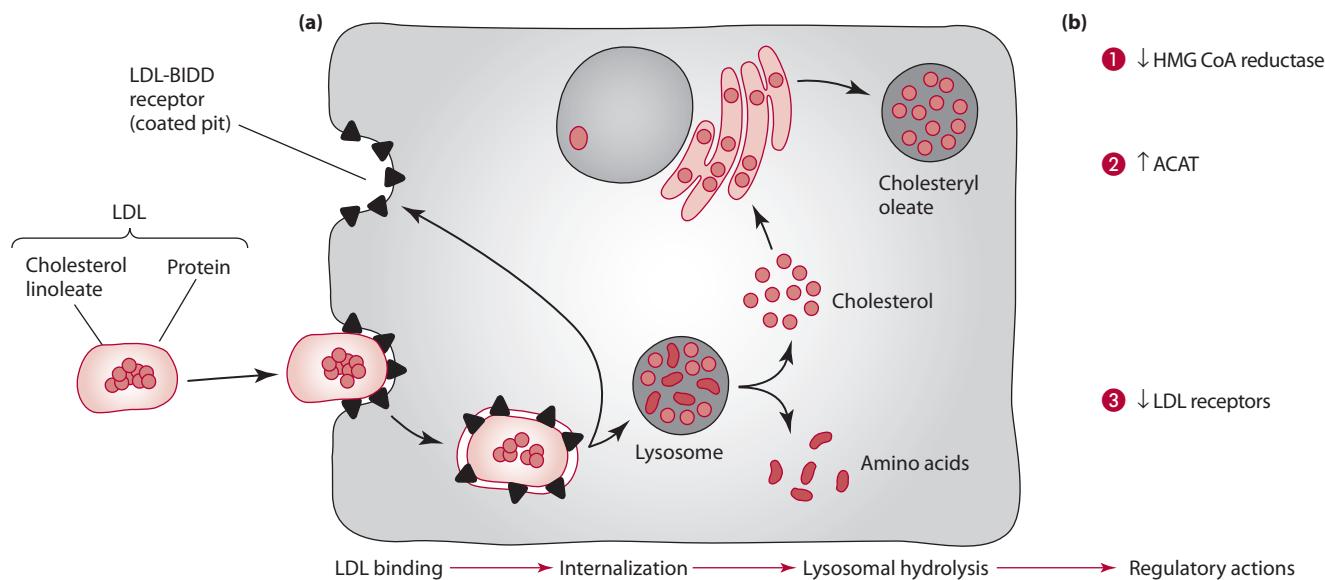


Figure 5.19 (a) Sequential steps in endocytosis of LDL leading to synthesis of cholesteryl ester. (b) Effect of cholesteryl ester on HMG CoA reductase, ACAT, and the concentration of LDL receptors in the cell.

- Domain 2, made up of 350 amino acids, is the possible location for the N-linked glycosylation that occurs during the maturation process of the receptor protein.
- Domain 3 is located immediately outside the plasma membrane and is the site of the O-linked glycosylation. This glycosylation, too, occurs during the maturation process of the receptor.
- Domain 4 is made up of 22 hydrophobic amino acids that, because of their affinity for lipids, are able to span the plasma membrane.
- Domain 5, the final domain, is the COOH terminal end of the protein and projects into the cytoplasm. This tail enables the receptors to move laterally, thereby mediating the clustering of the receptors in the coated pits.
- Class 1: No receptors are synthesized.
- Class 2: Precursors of the receptors are synthesized but then are not processed properly and fail to move into the Golgi apparatus.
- Class 3: The precursors for the LDL receptors are synthesized and processed, but the processing is faulty, preventing the receptors from binding LDL normally.
- Class 4: Mutations allow production of receptors that reach the surface of the cell and bind LDL but are unable to cluster in the coated pits.

Along with the delineation of the structure of the normal LDL receptor, knowledge of the structural defects existing in mutants has developed. Although a gene on chromosome 19 encodes the protein of the LDL receptor, the mutations of the gene are not always the same. How the normal functioning of the receptor is affected depends on what particular domains of the receptor have undergone mutation. The term *familial hypercholesterolemia* has been used for a variety of conditions that result in greatly elevated serum cholesterol levels and are caused by genetic metabolic defects. These defects can be either homozygous or heterozygous. The defects commonly involve dysfunctional or absent LDL receptors. Of the 110 familial hypercholesterolemia homozygotes studied, 10 different abnormal forms of the LDL receptors have been identified. These abnormalities can be divided into four classes:

Maturation of the LDL receptor precursor proteins, like that of other proteins synthesized on the endoplasmic reticulum of the cell, occurs in the cell's Golgi apparatus. There the LDL receptors are targeted for their final destination (see Chapter 1). Incomplete or improper processing can prevent the receptor from reaching its proper destination on the plasma membrane.

Relatively few people (1 in 1 million) are homozygous for familial hypercholesterolemia, but many people (1 in 500) carry one mutant gene for the disease. Knowing the mechanisms of the disease can be tremendously helpful in treating these individuals. There is little doubt that a causal relationship exists between hypercholesterolemia and the development of atherosclerosis.

Reducing serum cholesterol through drug therapy can cause the one normal gene in heterozygotes to increase transcription for LDL receptors, and serum cholesterol can thereby be normalized. Drug therapy includes both bile acid-binding resins, which increase fecal removal of cholesterol, and HMG CoA reductase inhibitors, which reduce cholesterol synthesis in the liver.

Many people who exhibit no clear-cut genetic defect also possess an inadequate number of LDL receptors. In this population, nutrition could be the environmental factor leading to decreased production of LDL receptors. A diet high in saturated fats and cholesterol appears to be one of the culprits.

The concentration of many cell receptors can be regulated. For example, the number of receptors present in target cells can be decreased if the concentration of an agonist circulating in the blood remains high for an extended period of time. This approach is called down-regulation.

To summarize the role of the LDL fraction in normal lipid metabolism, it can be thought of as a depositor of cholesterol and other lipids in peripheral cells that possess the LDL receptor. Cells targeted by LDL include the cells of the vascular endothelium. It therefore follows that high concentration and activity of LDL have implications in the etiology of cardiovascular disease.

High-Density Lipoprotein (HDL)

Opposing LDL's cholesterol-depositing role is the HDL fraction of serum lipoproteins. The function of HDL has been called reverse cholesterol transport. Figure 5.20 provides a description of the metabolism of HDL. An important function of HDL is to remove unesterified cholesterol from cells and other lipoproteins, where it may have accumulated, and return it to the liver to be excreted in the bile. Two key properties of HDL are necessary for this process to occur.

The first key property is HDL's ability to bind to receptors on both hepatic and extrahepatic cells. Receptors may be specific for HDL, but they also include the LDL receptor,

to which HDL can bind through its apoE component. In other words, the LDL receptor recognizes both apoE and apoB-100. Consequently, it is called the apoB, E receptor. The implication is that HDL can compete with LDL at its receptor site.

The second key property of HDL is mediated through its apoA-1 component, which stimulates the activity of the enzyme lecithin: cholesterol acyltransferase (LCAT). This enzyme forms cholesteryl esters from free cholesterol by catalyzing the transfer of fatty acids from the C-2 position of phosphatidylcholine to free cholesterol. The free cholesterol (recipient) substrate is derived from the plasma membrane of cells or surfaces of other lipoproteins. Cholesteryl esters resulting from this reaction can then exchange readily among plasma lipoproteins, mediated by a transfer protein called cholesteryl ester transfer protein (CETP). LCAT, by taking up free cholesterol and producing its ester form, thus promotes the net transfer of cholesterol out of non-hepatic cells and other lipoproteins. Cholesteryl esters can then be transported directly to the liver in association with HDL or indirectly by LDL, following CETP transfer from HDL to LDL. Recall that either lipoprotein can bind to LDL (apoB, E) receptors.

After the cholesterol esters are deposited in the liver cells, they are hydrolyzed by cholesteryl esterase, and the free cholesterol is excreted in the bile as bile salt (Figure 5.11). This process is the major route by which cholesterol is excreted from the body. The net effect of these properties of HDL is that cholesterol is retrieved from peripheral cells and other lipoproteins and returned, as cholesteryl ester, to the liver. This process is called

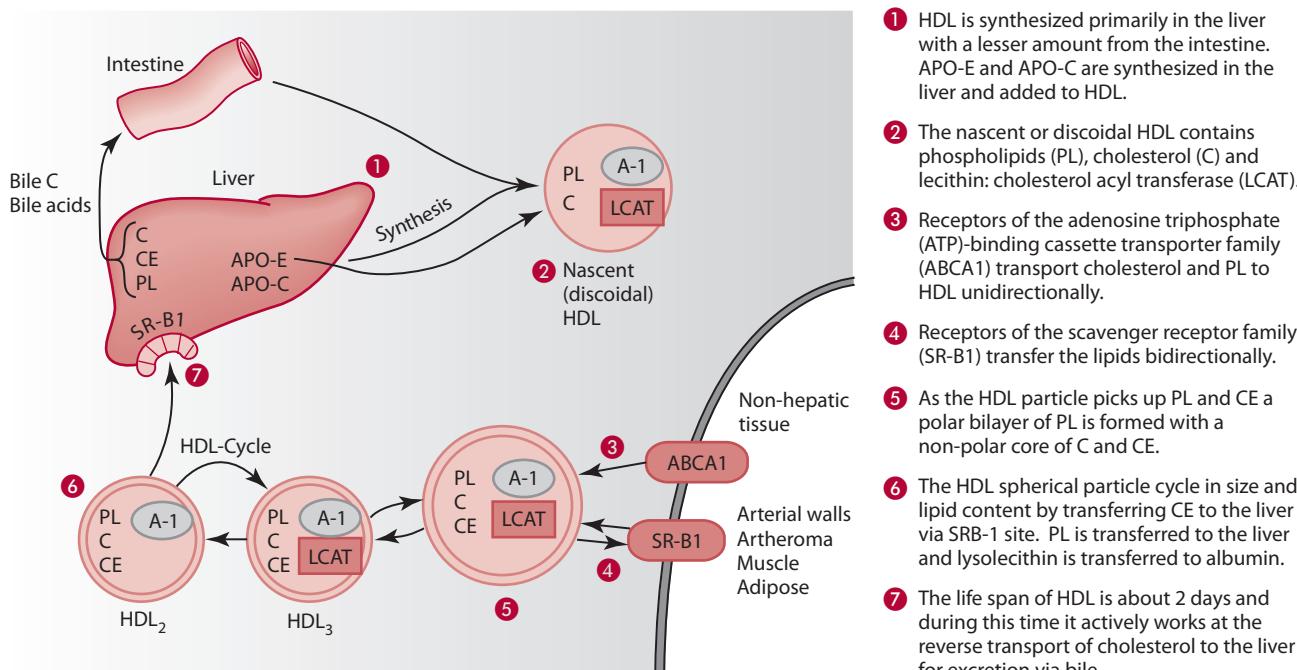


Figure 5.20 Reverse cholesterol transport.

reverse cholesterol transport. Its benefit to the cardiovascular system is that by reducing the amount of deposited cholesterol in the vascular endothelium, it also reduces the risk of fatty plaque formation and atherosclerosis. This delivery of cholesteryl esters to the liver presumably explains the correlation of high HDL levels with reduced risk of cardiovascular disease, a topic reviewed in the next section.

Lipids, Lipoproteins, and Cardiovascular Disease Risk

Atherosclerosis is a degenerative disease of vascular endothelium. The principal players in the atherogenic process are cells of the immune system and lipid material, primarily cholesterol and cholesteryl esters. An early response to arterial endothelial cell injury is an increased adherence of monocytes and T lymphocytes to the area of the injury. **Cytokines**, protein products of the monocytes and lymphocytes, mediate the atherogenic process by chemotactically attracting phagocytic cells to the area. Additional exposure to a high level of circulating LDL and the deposition and oxidative modification of cholesteryl esters further promote the inflammatory process. The process is marked by the uptake of LDL by phagocytic cells that become engorged with lipid, called foam cells. Phagocytic uptake is accelerated if the apoB component of the LDL is modified by oxidation. Lipid material, in the form of foam cells, may then infiltrate the endothelium. As lipid accumulates, the lumen of the blood vessel is progressively occluded. The deposited lipid, known to be derived from blood-borne lipids, is called fatty plaque. The pathophysiology of atherosclerosis has been reviewed [8] and is the subject of this chapter's Perspective.

Ever since plaque was found to be composed chiefly of lipids, an enormous research effort has been underway to investigate the possible link between dietary lipids and the development of atherosclerosis. The presumed existence of such a link has come to be known as the lipid hypothesis, which maintains that dietary lipid intake can alter blood lipid levels, which in turn initiate or exacerbate atherogenesis. The next section contains a brief account of the alleged involvement of certain dietary lipids and fatty acids, and of genetically acquired apolipoproteins, in atherogenesis.

CHOLESTEROL

At center stage in the lipid hypothesis controversy is cholesterol. The effects of dietary interventions designed to improve serum lipid profiles are often measured by the extent to which the interventions raise or lower serum

cholesterol. This reasoning is justified in that cholesterol is a major component of atherogenic fatty plaque, and many studies have linked cardiovascular disease risk to chronically elevated serum cholesterol levels. Receiving the greatest attention, however, is not so much the change in total cholesterol concentration but how the cholesterol is distributed between its two major transport lipoproteins, LDL and HDL. Because cholesterol is commonly and conveniently quantified in clinical laboratories, assays can be used to establish LDL:HDL ratios by measuring the amount of cholesterol in each of the two fractions. Assayed cholesterol associated with the LDL fraction is designated LDL-C by laboratory analysts, and cholesterol transported in the HDL fraction is designated HDL-C.

Because maintaining relatively low serum levels of LDL and relatively high levels of HDL (a low LDL:HDL ratio) supports wellness, the concept of "good" and "bad" cholesterol emerged. The "good" form is the cholesterol associated with HDL, and the "bad" form is the cholesterol transported as LDL. It is important to understand, however, that cholesterol itself is not "good" or "bad"; rather, it serves as a proxy for the relative concentrations of LDL and HDL, ratios of which can indeed be good or bad. LDL:HDL ratios are, in fact, determined more reliably by measurements other than cholesterol content. For example, immunological methods for quantifying apoB (the major LDL apoprotein) and apoA-1 (the primary HDL apoprotein) are now widely used. Ratios of apoA to apoB then serve as an indicator of cardiovascular disease risk, with risk decreasing as the ratio decreases. Approximately one molecule of the lipoprotein (apoB or apoA-1) is associated with each lipoprotein particle.

ApoB in the serum is made up of apoB-48, made in the intestinal cell, and ApoB-100, made in the liver. Most of the apoB present in the serum is apoB-100. This lipoprotein is found in VLDL, IDL, and LDL. The total moles of apoB present in the serum indicate the number of potentially atherogenic particles.

ApoA-1 is the major apoprotein in the HDL particles that are part of the reverse cholesterol system. HDL particles are antiatherogenic. They also have anti-inflammatory and antioxidant properties. A recent review provides more detailed information about the use of the apoB:apoA-1 ratio for assessing the cardiovascular risk [9].

Associated with lower levels of LDL and higher levels of HDL is the level of total cholesterol, which is often the focus of cardiovascular risk. Among the reasons for cholesterol's "bad press" in connection with cardiovascular disease is the fact that cholesterol, and especially cholesteryl esters, are major components of fatty plaque. Contrary to widespread belief, changing the amount of cholesterol in the diet has only a minor influence on blood cholesterol concentration in most people. This is because compensatory mechanisms are engaged, such as HDL activity in scavenging excess cholesterol and the down-regulation of

cholesterol synthesis by dietary cholesterol (discussed in the section “Synthesis of Cholesterol” in this chapter). It is well known, however, that certain individuals respond strongly, and others weakly, to dietary cholesterol (hyper- and hyporesponders). This phenomenon, which may have a genetic basis, is further complicated by the observation that considerable within-person variability exists independent of diet, a fact that clearly confounds the results of intersubject studies.

Several mechanisms may be considered when trying to account for differences in individual responses to dietary cholesterol, including differences in:

- absorption or biosynthesis
- formation of LDL and its receptor-mediated clearance
- rates of LDL removal and excretion

These considerations have been extensively reviewed [10].

SATURATED AND UNSATURATED FATTY ACIDS

Research examining the influence of various kinds of fatty acids on cardiovascular disease risk has focused on the effect that each kind has on serum cholesterol levels. The literature dealing with the effect of dietary fats containing primarily saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), or *trans* fatty acids is as extensive as that related to consumption of cholesterol itself. Early research results generally led to the conclusion that SFAs are hypercholesterolemic, and PUFAs are hypocholesterolemic. Furthermore, MUFAs were assumed to be neutral, neither increasing nor lowering serum cholesterol.

Current research focuses not so much on total cholesterol effects but rather on how LDL-C:HDL-C ratios are shifted by the test lipids. For example, studies have shown diets rich in MUFA to be as effective as PUFA-rich diets in lowering LDL cholesterol and triacylglycerols without significant change in HDL [11,12]. How the position of the double bonds in PUFA (n-3 vs. n-6 species), and their *cis* and *trans* isomerism, relates to lipoprotein ratio effects has also been a focus of interest. The effect of *trans* fats is discussed in the next section of this chapter.

The understanding of the role of dietary intake of lipids in cardiovascular disease risk has changed over time [13,14]. Consumption of the following lipids shows a *positive* correlation with the risk of cardiovascular disease (CVD), primarily from a hypercholesterolemic effect or from unfavorable shifts in LDL-C:HDL-C ratios:

- total fat
- saturated fatty acids
- cholesterol
- *trans* fat

Consumption of the following lipids shows a *negative* correlation with CVD risk, primarily from a hypocholesterolemic effect or from favorable shifts in LDL:HDL ratios:

- Monounsaturated fatty acids (if adjustments are made for cholesterol and saturated fatty acids consumed).
- Polyunsaturated fatty acids (if adjusted for cholesterol and saturated fatty acids). Both n-3 and n-6 types are effective. The linoleic acid (18:2 n-6) content in adipose tissue was found to be inversely associated with CVD risk. The greater the content of 18:2 n-6, the lower the risk for CVD.
- n-3 fatty acids. The n-3 PUFAs exert antiatherogenic properties by various mechanisms, including these:
 - Interference with platelet aggregation, in part by inhibiting thromboxane (TXA₂) production. Inhibition is thought to be caused by fatty acids displacement of the TXA₂ precursor, arachidonic acid, from platelet phospholipid stores. Eicosapentaenoic acid (EPA; 20:5 n-3), docosahexaenoic acid (DHA; 20:6 n-3), and α-linolenic acid (18:3 n-3) exerted similar antiaggregatory effects [14].
 - Reduction in the release of proinflammatory cytokines from cells involved in fatty plaque formation (see this chapter’s Perspective, “The Role of Lipids and Lipoproteins in Atherogenesis”).
 - Sharp reduction (25%–30%) in serum triacylglycerol concentration. α-linoleate was less effective than EPA or DHA, and plant n-3 fatty acids were generally less effective than marine n-3 fatty acids in their capacity to reduce triacylglycerols [15,16].

The potential risk of CVD is actually more complicated than what is implied by listing positive and negative correlates. It involves a combination of genetics, dietary factors, exercise, and other lifestyle determinants. For instance, the cholesterolemic response to individual fatty acids, even those within a single fatty acid class, is heterogeneous. This heterogeneity is particularly noticeable among the long-chain saturated fatty acids. Strong evidence indicates that lauric (12:0), myristic (14:0), and palmitic (16:0) acids are all hypercholesterolemic, specifically raising LDL-C, with myristic acid (14:0) being the most potent in this respect. On the other hand, stearic acid (18:0), neutral in its effect, in fact is reputed to reduce levels of total cholesterol and LDL-C when compared to other long-chain saturated fatty acids. Therefore, stearic acid should not be grouped with shorter-chain SFAs with respect to LDL-C effects. Oleic acid (18:1) and linoleic acid (18:2 n-6) are more hypocholesterolemic than 12:0 and 16:0 fatty acids. Linoleate (18:2 n-6) is the more potent of the two, independently lowering total and LDL cholesterol [17].

Despite years of investigation, the mechanism by which hypercholesterolemic fatty acids exert their atherogenic effects has not been conclusively defined. However, they

have been alleged to operate in one or more of the following ways:

- by suppressing the excretion of bile acids, thereby lowering lipid absorption
- by enhancing the synthesis of cholesterol and LDL, either by reducing the degree of control exerted on the regulatory enzyme HMG CoA reductase or by affecting apoB synthesis
- by retarding LCAT activity or receptor-mediated uptake of LDL
- by acting as regulators of gene expression

TRANS FATTY ACIDS

Double-bonded carbon atoms can exist in either a *cis* or a *trans* orientation, as described in Figure 5.1. Most natural fats and oils contain only *cis* double bonds. The much smaller number of naturally occurring *trans* fats are found mostly in the fats of ruminants, for example, in milk fat, which contains 4% to 8% *trans* fatty acids. Much larger amounts are found in certain margarines and margarine-based products, shortenings, and frying fats as a product of the partial hydrogenation of PUFA. The process of hydrogenation gives the product a higher degree of hardness (remaining solid at room temperature) and plasticity (spreadability), which is more desirable to both the consumer and the food manufacturer. Frying oils have also been hydrogenated to enhance their stability. In the hydrogenation process, as hydrogen atoms are catalytically added across double bonds, electronic shifts take place that cause remaining, unhydrogenated *cis* double bonds to revert to a *trans* configuration that is energetically more stable. Current food labeling regulations and public concern have greatly reduced the amount of *trans* fatty acids available in the food supply.

The most abundant *trans* fatty acids in the diet are elaidic acid (Figure 5.1) and its isomers, which are of an 18:1 structure, though 18:2 *trans* fatty acids are also found. It has been reported that diets rich in these fatty acids are as hypercholesterolemic as saturated fatty acids [18]. In fact, serum lipid profiles following feeding of a diet high in *trans* fatty acids may be even more unfavorable than those produced by saturated fatty acids, because not only are total cholesterol and LDL cholesterol levels elevated, but the HDL cholesterol level is lowered. The study cited was criticized for using *trans* elaidic acids obtained by a process not typical of the hydrogenation of margarines and shortenings and also for including uncharacteristically large dietary amounts of the *trans* fatty acids in the study diet. However, reports from subsequent studies confirm that *trans* fat consumption elevates serum LDL-C while decreasing HDL-C and also raises total cholesterol:HDL-C ratios [19]. Another confirmatory investigation, conducted on a large group of healthy women over an 8-year period, matched *trans* fat intake with the

incidence of nonfatal myocardial infarction or death from coronary heart disease [20]. Although the study indicated a positive correlation between *trans* fat intake and coronary heart disease, it did not escape criticism because the data were obtained by consumer questionnaires rather than by a randomized controlled study in which intake could be precisely monitored by researchers [21]. Furthermore, the *trans* fatty acids in the foods consumed by the subjects varied considerably, and a clear-cut dose-response relationship could not be demonstrated [22]. A more recent report from the Nurses' Health Study measured the amount of *trans* fatty acids in the erythrocytes of subjects at baseline and followed subjects for a 6-year period for nonfatal myocardial infarctions and coronary heart disease (CHD) deaths. Erythrocyte *trans* fatty acids were also measured in control subjects. This study used biomarkers for the *trans* fatty acids rather than food intake questionnaires to estimate the level of intake of *trans* fatty acids. The study showed a threefold increase in the risk of CHD between the bottom quartile of erythrocyte *trans* fatty acids and the top quartile [23].

Some reports exonerate *trans* fat of its alleged hypercholesterolemic properties. In a randomized crossover study involving hypercholesterolemic subjects, replacing butter with margarine in a low-fat diet actually lowered LDL-C and apolipoprotein B by 10%, while HDL-C and apolipoprotein A levels were unaffected [24].

Current public health guidelines recommend keeping the intake of *trans* fatty acids as low as is reasonable. Reducing *trans* fatty acid intake to zero is impossible, because *trans* fats occur naturally. Labeling requirements for *trans* fatty acids make the consumer more aware of foods that contain them, and more and more foods are being labeled as *trans* fatty acid free. The adverse findings on *trans* fats represent an about-face from the longstanding nutrition dogma that unsaturated fats are invariably preferred over saturated fats.

LIPOPROTEIN A

In the 1960s, a genetic variant of LDL was discovered in human serum. The particle differs from normal LDL in that it is attached to a unique marker protein of high molecular weight (513,000 D). The marker protein is currently referred to as apolipoprotein a, or apo(a), and the complete lipoprotein particle is called lipoprotein a, or Lp(a).

At the time of the discovery of Lp(a), it was evident that not all people had the lipoprotein in their serum. Also, in many of those who did, its concentration was very low compared with other lipoproteins. Consequently, it was dismissed as having little importance. However, interest in Lp(a) was renewed during the following two decades, when numerous studies suggested a positive correlation between Lp(a) concentration and atherosclerotic disease.

Structurally, Lp(a) is assembled from LDL and the apo(a) protein. The LDL component of the complex possesses apoB-100 as its only protein component. The LDL portion of the particle is linked to apo(a) through a disulfide bond connecting the two proteins apo(a) and apoB-100. A strong structural homology (similar amino acid sequence) has recently been discovered between apo(a) and plasminogen. Plasminogen is the inactive precursor of the enzyme plasmin, which dissolves blood clots by its hydrolytic action on fibrin. This discovery has stimulated extensive research into the genetics, metabolism, function, and clinical significance of Lp(a).

The physiological function of Lp(a) is not yet defined with certainty, although it is tempting to speculate that its role may be linked to the two functional systems from which the particle was derived: a lipid transport system and the blood-clotting system. It has been proposed that Lp(a) may bind to fibrin clots by its plasminogen-like apo(a) and therefore may deliver cholesterol to regions of recent injury and wound healing.

Reviews of Lp(a) cite numerous epidemiological studies [25] that show a positive correlation between Lp(a) concentration and premature myocardial infarction, which occurs when blood vessels in the heart are blocked by clot formation. This finding has led to the conclusion that Lp(a) may represent an independent genetic risk for atherosclerotic disease. Unfortunately, blood levels of the lipoprotein do not respond to dietary intervention and respond only very weakly to lipid-lowering drugs. One of the many open questions is whether Lp(a) is linked to atherosclerosis over an extended period of time because of its lipoprotein properties or instead plays a role in the sudden development of a clot due to the binding of its plasminogen-like apo(a) component to fibrin. Perhaps both mechanisms apply.

APOLIPOPROTEIN E

Recall that the term *apolipoprotein* refers to the protein moiety of a lipoprotein. Studies have shown that one apolipoprotein, apolipoprotein E (apoE), may have a role in the etiology of atherosclerosis. As shown in Table 5.4, apoE is a structural component of VLDL, HDL, chylomicrons, and chylomicron remnants. The most important physiological function of apoE is that it is the component recognized by the LDL receptor-related protein (also referred to as the postulated apoE receptor) and the LDL receptor (Figure 5.18). By way of interaction with these receptors, apoE mediates the uptake of apoE-containing lipoproteins into the liver.

A common polymorphism in the apoE gene codes for three isoforms: apoE2, -E3, and -E4. The role of these isoforms in CHD has been studied extensively. They are genetically encoded by three alleles, E2, E3, and E4, respectively. A single individual inherits one allele from

each parent and therefore will express one of the following six possible phenotypes: E2,2; E3,2; E3,3; E3,4; E4,2; or E4,4. The effects of apoE polymorphism on plasma lipids and a predisposition to cardiovascular disease have been well studied [26, 27]. Meta-analysis from 48 studies demonstrates that E4 phenotypes confer an increased risk of developing cardiovascular disease. This increase is not fully understood but is apparently caused by elevated LDL-C serum level and lower HDL-C levels. The apoE phenotype alone is associated with a stepwise increase in LDL-C (cholesterol contained within the LDL fraction). LDL-C increased in the order of phenotypes E3,2 < E3,3 < E4,3. This relationship is independent of the ratio of polyunsaturated to saturated fats consumed [28].

Based on the link between the E4 allele and a predisposition to cardiovascular disease, the allele is considered to be a predictor of latent atherogenesis. A longitudinal study of elderly men conducted over a 5-year period revealed that the E4 allele was twice as common among men who died of coronary heart disease during the study period as it was among those who did not [29].

Integrated Metabolism in Tissues

CATABOLISM OF TRIACYLGLYCEROLS AND FATTY ACIDS

The complete hydrolysis of triacylglycerols yields glycerol and three fatty acids. In the body, this hydrolysis occurs largely through the activity of lipoprotein lipase of vascular endothelium in non-hepatic tissue and through an intracellular lipase that is active both in the liver and particularly active in adipose tissue. The glycerol portion can be used for energy by the liver and by other tissues having activity of the enzyme glycerokinase, through which glycerol is converted to glycerol phosphate. Glycerol phosphate can enter the glycolytic pathway at the level of dihydroxyacetone phosphate, from which point either energy oxidation or gluconeogenesis can occur (review Figure 3.17).

Fatty acids are a very rich source of energy, and on an equal-weight basis they surpass carbohydrates in this property. This occurs because fatty acids exist in a more reduced state than that of carbohydrate and therefore undergo a greater extent of oxidation en route to CO_2 and H_2O . Many tissues are capable of oxidizing fatty acids by way of a mechanism called β -oxidation, described below. When it enters the cell of the metabolizing tissue, the fatty acid is first activated by coenzyme A, an energy-requiring reaction catalyzed by cytoplasmic fatty acyl CoA synthetase (Figure 5.21). The reaction consumes two high-energy phosphate bonds to yield AMP. This is equivalent to using two ATPs. The pyrophosphates that

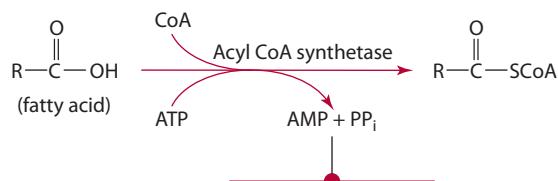


Figure 5.21 Activation of fatty acid by coenzyme A.

are produced are quickly hydrolyzed, which ensures that the reaction is irreversible.

Mitochondrial Transfer of Acyl CoA

The oxidation of fatty acids occurs within the mitochondrion. Short-chain fatty acids can pass directly into the mitochondrial matrix. Long-chain fatty acids and their CoA derivatives are incapable of crossing the inner mitochondrial membrane (but can cross the permeable outer membrane), so a membrane transport system is necessary. The carrier molecule for this system is carnitine (see Chapter 9), which can be synthesized in humans from lysine and methionine and is found in high concentration in muscle. The activated fatty acid (acyl CoA) is joined covalently to carnitine at the cytoplasmic side of the mitochondrial membrane by the transferase enzyme carnitine acyltransferase I (CAT I). A second transferase, carnitine acyltransferase II (CAT II), located on the inner face of the inner membrane, releases the fatty acyl CoA and carnitine into the matrix (Figure 5.22).

β-Oxidation of Fatty Acids

The oxidation of the activated fatty acid in the mitochondrion occurs through a cyclic degradative pathway by which two-carbon units in the form of acetyl CoA are cleaved one by one from the carboxyl end. The reactions of β-oxidation are summarized in Figure 5.23. The activated palmitoyl CoA is acted upon by the enzyme acyl CoA dehydrogenase to produce a double bond between the α- and β-carbons. There are four such dehydrogenases, each specific to a range of chain lengths. The

unsaturated acyl CoA adds a molecule of water to form a β-hydroxyacyl CoA with the aid of the enzyme enoyl CoA hydratase, sometimes called crotonase. The β-hydroxy group is then oxidized to a ketone by the NAD⁺ requiring enzyme β-hydroxyacyl CoA dehydrogenase, producing a NADH that can go into the electron transport chain to produce about 3 ATPs. The β-ketoacyl CoA is cleaved by acyl transferase (also called thiolase), resulting in the insertion of another CoA and cleavage at the β-carbon. The products of this reaction are acetyl CoA (which enters the TCA cycle for further oxidation) and a saturated CoA-activated fatty acid that has two fewer carbons than the original fatty acid. The entire sequence of reactions is repeated, with two carbons being removed with each cycle.

ENERGY CONSIDERATIONS IN FATTY ACID OXIDATION

The activation of a fatty acid requires two high-energy bonds per mole of fatty acid oxidized. Each cleavage of a saturated carbon-carbon bond yields five ATPs, two by oxidation of FADH₂ and three by oxidation of NADH by oxidative phosphorylation. The acetyl CoAs produced are oxidized to CO₂ and water in the TCA cycle, and for each acetyl CoA oxidized, 12 ATPs (or their equivalent) are produced (see Chapter 3). Using the example of palmitate (16 carbons), we can summarize the yield of ATP as follows:

7 carbon-carbon cleavages	$7 \times 5 = 35$
8 acetyl CoAs oxidized	<u>$8 \times 12 = 96$</u>
Total ATPs produced	131
2 ATPs for activation	<u><u>-2</u></u>
Net ATPs	129

Nearly one-half of dietary and body fatty acids are unsaturated and provide a considerable portion of lipid-derived energy. They are catabolized by β-oxidation in the mitochondrion in nearly the same way as their saturated counterparts, except that one fatty acyl CoA dehydrogenase reaction is not required for each double bond present. This is because the double bond introduced into the saturated fatty acid by the reaction occurs naturally in unsaturated fatty acids. However, the specificity of the enoyl CoA hydratase reaction requires that the double bond be between the second and third carbon in order for the hydration to take place, and the “natural” double bond may not occupy the Δ² position. For example, after three cycles of β-carbon oxidation, the position of the double bond in what was originally a Δ⁹ monounsaturated fatty acid will occupy a Δ³ position. Figure 5.24 shows 18:1 Δ⁹ undergoing three cycles of β-oxidation. At the end of the third cycle, the fatty acid is a Δ³ fatty acid. The presence of a specific enoyl-CoA-isomerase then shifts the double bond from a *cis* Δ³ to *trans* Δ², allowing the hydrase and subsequent reactions to proceed. The oxidation of an unsaturated

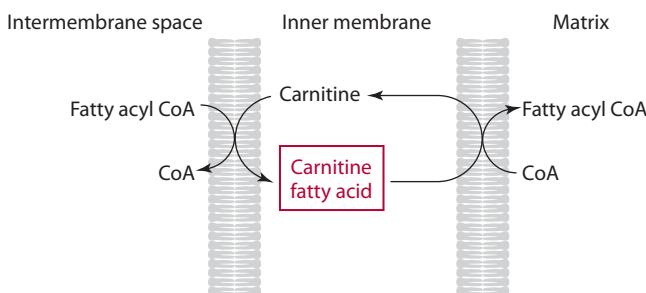
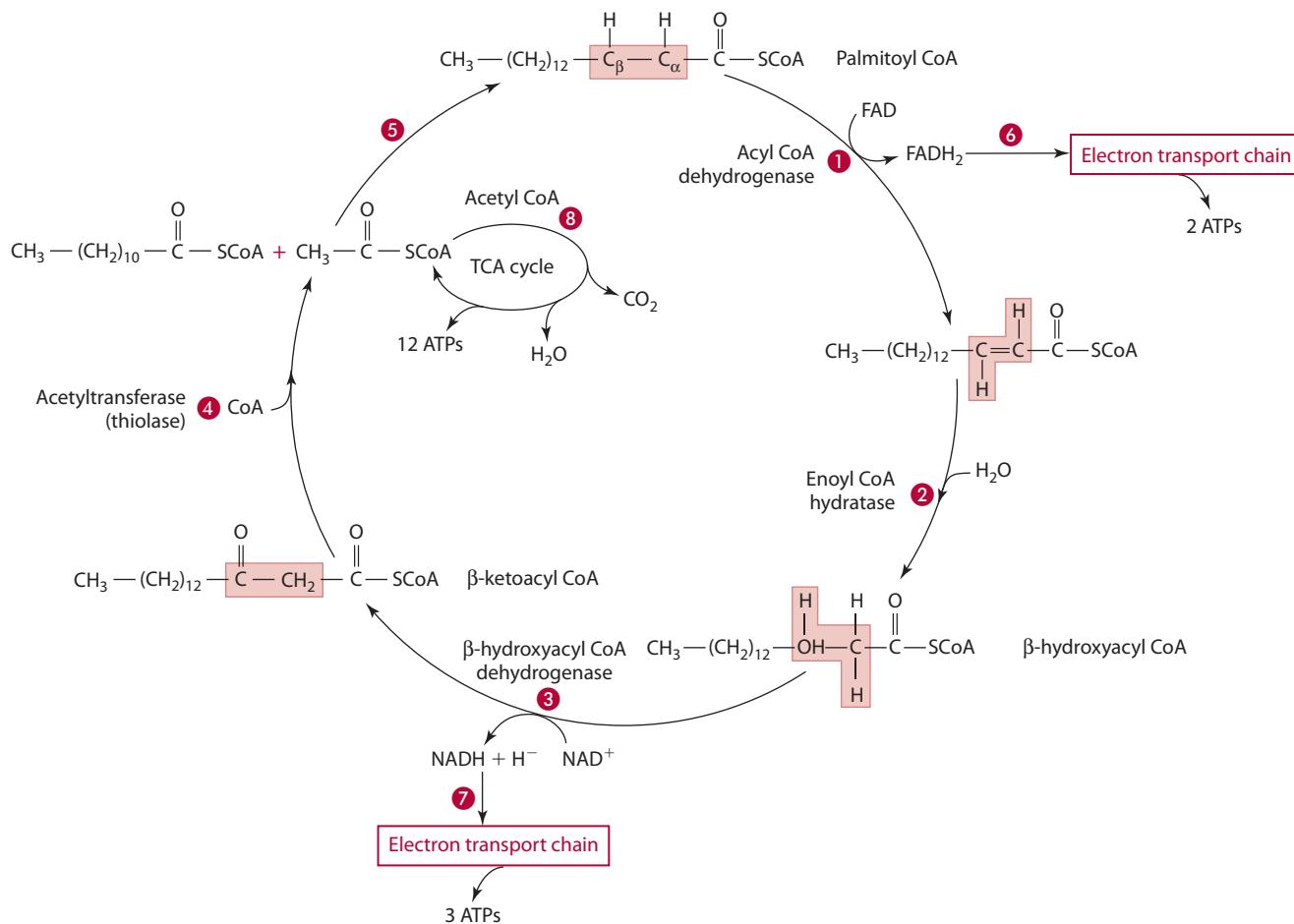


Figure 5.22 Membrane transport system for transporting fatty acyl CoA across the inner mitochondrial membrane.



- ① The formation of a double bond between the α- and β-carbons is catalyzed by acyl CoA dehydrogenase. There are four such dehydrogenases, each specific to a range of chain lengths.
- ② The unsaturated acyl CoA adds a molecule of water. The reaction is catalyzed by enoyl CoA hydratase, sometimes called crotonase.
- ③ The β-hydroxy group is oxidized to the ketone by the NAD⁺—requiring enzyme β-hydroxyacyl CoA dehydrogenase.
- ④ The β-ketoacyl CoA is cleaved by acyl transferase (also called thiolase), resulting in the insertion of CoA and the cleavage at the β-carbon. The products of this reaction are acetyl CoA and a saturated CoA-activated fatty acid having two fewer carbons than the original fatty acid.
- ⑤ This entire sequence of reactions is repeated, with two carbons being removed with each cycle.
- ⑥ A FADH₂ moves into the electron transport system and produces 2 ATPs.
- ⑦ A NADH is produced and moves into the electron transport system to produce about 3 ATPs.
- ⑧ Each acetyl CoA is further oxidized by the TCA cycle to produce 12 ATPs.

Figure 5.23 The mitochondrial β-oxidation of an activated fatty acid using palmitate as an example.

fatty acid results in somewhat less energy production than oxidation of a saturated fatty acid of the same chain length, because for each double bond present one FADH₂-producing fatty acyl CoA dehydrogenase reaction is bypassed, resulting in two fewer ATPs.

Although most fatty acids metabolized are composed of an even number of carbon atoms, small amounts of fatty acids having an odd number of carbon atoms are also used for energy. β-oxidation occurs as described, with the liberation of acetyl CoA until a residual propionyl CoA remains. The subsequent oxidation of propionyl CoA requires reactions that use the vitamins biotin and B₁₂ in a coenzymatic

role (Figure 5.25). Because the succinyl CoA formed in the course of these reactions can be converted into glucose, the odd-numbered carbon fatty acids are uniquely glucogenic among all the fatty acids.

FORMATION OF KETONE BODIES

In addition to its direct oxidation through the TCA cycle, acetyl CoA may follow other catabolic routes in the liver, one of which is the pathway by which the so-called **ketone bodies** (acetoacetate, β-hydroxybutyrate, and acetone) are formed. Acetoacetate and β-hydroxybutyrate

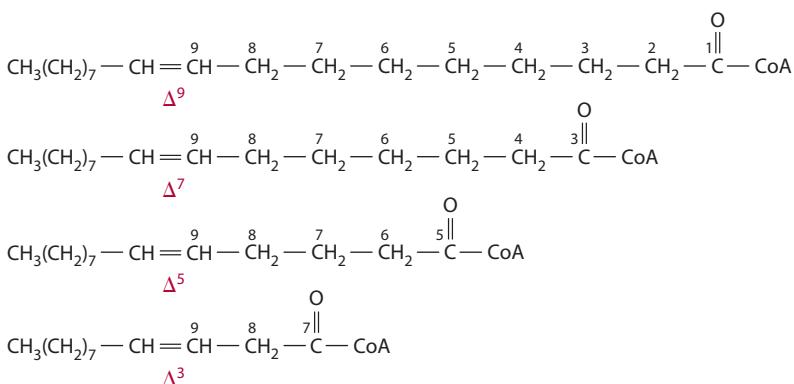


Figure 5.24 Sequential β -oxidation of oleic acid, showing the location of the double bond using the Δ nomenclature system. Numbers above the carbons represent the original carbon numbers of oleic acid.

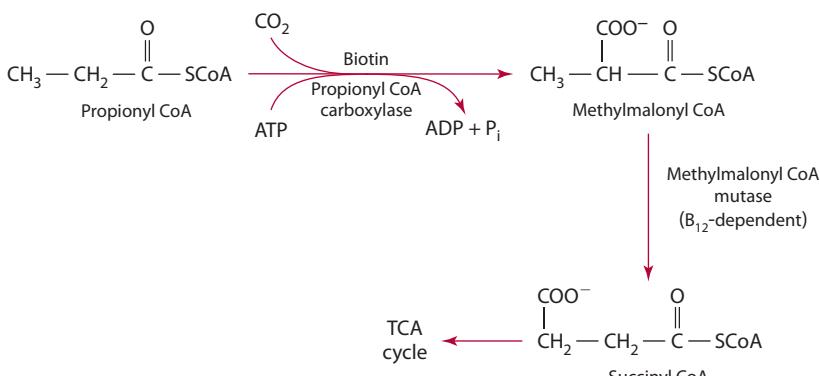


Figure 5.25 Oxidation of propionyl CoA.

are not oxidized further in the liver but instead are transported by the blood to peripheral tissues, where they can be converted back to acetyl CoA and oxidized through the TCA cycle. The steps in ketone body formation occur as shown in Figure 5.26. The reversibility of the β -hydroxybutyrate dehydrogenase reaction, together with enzymes present in extrahepatic tissues that convert acetoacetate to acetyl CoA (shown by broken arrows in Figure 5.26) reveals how the ketone bodies can serve as a source of fuel in these tissues.

Ketone body formation is actually an “overflow” pathway for acetyl CoA use, providing another way for the liver to distribute fuel to peripheral cells. Normally, the concentration of the ketone bodies in the blood is very low, but it may reach very high levels in situations of accelerated fatty acid oxidation combined with low carbohydrate intake or impaired carbohydrate use.

Such a situation would occur in diabetes mellitus, starvation, or simply a very low carbohydrate diet. Recall from Chapter 3 that for the TCA cycle to function, the supply of four-carbon units must be adequate. These intermediates are formed mainly from pyruvate (formed during glycolysis). Without the oxidation of glucose, the supply of carbohydrate is inadequate and thus, the pool of oxaloacetate, with which the acetyl CoA normally combines for oxidation in the TCA cycle, is reduced. As carbohydrate

use diminishes, oxidation of fatty acids accelerates to provide energy through the production of TCA cycle substrates (acetyl CoA). This shift to fat catabolism, coupled with reduced oxaloacetate availability, results in an accumulation of acetyl CoA. As would be expected, a sharp increase in ketone body formation follows, resulting in the condition known as ketosis. Ketosis can be dangerous because it can disturb the body’s acid-base balance (two of the ketone bodies are, in fact, organic acids). However, the liver’s ability to deliver ketone bodies to peripheral tissues such as the brain and muscle is an important mechanism for providing fuel in periods of starvation. In short, it is the lesser of two evils.

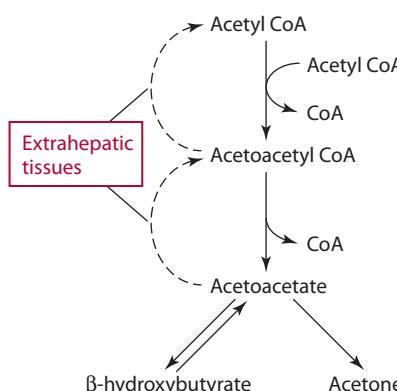


Figure 5.26 Steps in ketone body formation.

CATABOLISM OF CHOLESTEROL

Unlike the triacylglycerols and fatty acids, cholesterol is not an energy-producing nutrient. Its four-ring core structure remains intact in the course of its catabolism and is eliminated as such through the biliary system, as described earlier in this chapter. Cholesterol, primarily in the form of its ester, is delivered to the liver chiefly in the form of chylomicron remnants, as well as in the form of LDL-C and HDL-C. The cholesterol that is destined for excretion either is hydrolyzed by esterases to the free form, which is secreted directly into the bile canaliculi, or it is first converted into bile acids before entering the bile. It is estimated that neutral sterol, most of which is cholesterol, represents about 55%, and bile acids and their salts about 45%, of total sterol excreted.

The key metabolic changes in the cholesterol-to-bile acid transformation are:

- reduction in the length of the hydrocarbon side chain at C-17
- addition of a carboxylic acid group on the shortened chain
- addition of hydroxyl groups to the ring system of the molecule

The effect of these reactions is to enhance the water solubility of the sterol, facilitating its excretion in the bile. Cholic acid, whose structure is shown in Figure 5.11, has hydroxyl groups at C-7 and C-12 in addition to the C-3 hydroxyl of the native cholesterol. The other major bile acids differ from cholic acid only in the number of hydroxyls attached to the ring system. For example, chenodeoxycholic acid has hydroxyls at C-3 and C-7, deoxycholic acid at C-3 and C-12, and lithocholic acid at C-3 only. Other bile acids are formed from the conjugation of these compounds with glycine or taurine, which attaches through the carboxyl group of the steroid. These reactions are shown in Figure 5.11.

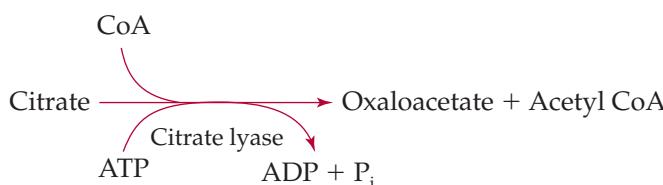
Recall that the enterohepatic circulation can return absorbed bile salts to the liver. Bile salts returning to the liver from the intestine repress the formation of an enzyme that catalyzes the rate-limiting step in the conversion of cholesterol into bile acids. If the bile salts are prevented from returning to the liver, the activity of this enzyme increases, stimulating the conversion of cholesterol to bile acids and leading to their excretion. The removal of bile salts is exploited therapeutically in treating hypercholesterolemia by using unabsorbable, cationic resins that bind bile salts in the intestinal lumen and prevent them from returning to the liver.

SYNTHESIS OF FATTY ACIDS

Aside from linoleic acid and α -linolenic acid, which are essential and must be acquired from the diet, the body is capable of synthesizing fatty acids from simple precursors. The basic process involves the sequential assembly

of a “starter” molecule of acetyl CoA with units of malonyl CoA, the CoA derivative of malonic acid. Ultimately, however, all the carbons of a fatty acid are contributed by acetyl CoA, because malonyl CoA is formed from acetyl CoA and CO_2 . This reaction occurs in the cytoplasm. It is catalyzed by acetyl CoA carboxylase, a complex enzyme containing biotin as its prosthetic group. The role of biotin in **carboxylation reactions** (such as this one), which involve the incorporation of a carboxyl group into a compound, is discussed in Chapter 9. ATP furnishes the driving force to attach the new carboxyl group to acetyl CoA (Figure 5.27).

Nearly all the acetyl CoA formed in metabolism occurs in the mitochondria. It is formed there from pyruvate oxidation, from the oxidation of fatty acids, and from the degradation of the carbon skeletons of some amino acids (see Chapter 6). The synthesis of fatty acids is localized in the cytoplasm, but acetyl CoA as such is unable to pass through the mitochondrial membrane. The major mechanism for the transfer of acetyl CoA to the cytoplasm is by way of its passage across the mitochondrial membrane in the form of citrate. In the cytoplasm, citrate lyase converts the citrate to oxaloacetate and acetyl CoA. This reaction, shown here, is essentially the reversal of the citrate synthetase reaction of the TCA cycle, except that it requires expenditure of ATP.



The enzymes involved in fatty acid synthesis are arranged in a complex called the fatty acid synthase system and is found in the cytoplasm. Key components of this complex are the acyl carrier protein (ACP) and the condensing enzyme (CE), both of which possess free —SH groups to which the acetyl CoA and malonyl CoA building blocks attach. ACP is structurally similar to CoA (Figure 9.18). Both possess a 4'-phosphopantetheine component (pantothenic acid coupled through -alanine to thioethanolamine) and phosphate. The thioethanolamine contributes the free —SH group to the complex. The free —SH of the condensing enzyme is contributed by the amino acid cysteine.

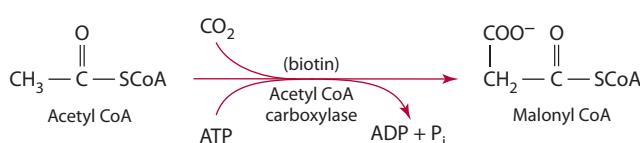


Figure 5.27 Formation of malonyl CoA from acetyl CoA and CO_2 (carboxylation reaction).

Before the actual steps in the elongation of the fatty acid chain can begin, the two sulphydryl groups must be “loaded” correctly with malonyl and acetyl groups. Acetyl CoA is transferred to ACP, with the loss of CoA, to form acetyl ACP. The acetyl group is then transferred again to the —SH of the condensing enzyme, leaving available the ACP—SH, to which malonyl CoA attaches, again with the loss of CoA. This loading of the complex can be represented as in Figure 5.28. The extension of the fatty acid chain then proceeds through the following sequential steps, which also are shown schematically in Figure 5.29 along with the enzymes and cofactors catalyzing the reactions. The enzymes catalyzing these reactions are also part of the fatty acid synthase complex along with ACP and CE.

The first step is the coupling of the carbonyl carbon of the acetyl group to the C-2 of malonyl ACP with the elimination of the malonyl carboxyl group as CO_2 . The β -ketone is then reduced, with NADPH serving as hydrogen donor. This alcohol is dehydrated, yielding a double bond. The double bond is reduced to butyryl-ACP, again with NADPH acting as reducing agent. The butyryl group is transferred to the CE, exposing the ACP sulphydryl site, which accepts a second molecule of malonyl CoA. A second condensation reaction takes place, coupling the butyryl group on the CE to C-2 of the malonyl ACP. The six-carbon chain is then reduced and transferred to CE in a repetition of steps 2 through 5. A third molecule of malonyl CoA attaches at ACP—SH, and so forth. The completed fatty acid chain is hydrolyzed from the ACP without transfer to the CE. The normal product of the fatty acid synthase system is palmitate, 16:0. It can in turn be lengthened by fatty acid elongation systems to stearic acid, 18:0, and even longer saturated fatty acids. Elongation occurs by the addition of two-carbon units at the carboxylic acid end of the chain. Furthermore, by **desaturation** reactions, palmitate and stearate can be converted to their corresponding Δ^9 monounsaturated fatty acids, palmitoleic acid (16:1) and oleic acid (18:1), respectively. Fatty acid desaturation reactions are catalyzed by enzymes referred to as mixed-function oxidases, so called because two different substrates are oxidized: the fatty acid (by removal of hydrogen atoms to form the new double bond) and NADPH. Oxygen is the terminal hydrogen and electron acceptor to form H_2O . Note that most of the acetyl CoA is produced in the mitochondria from the oxidation of

pyruvate. For fatty acid synthesis to take place, the acetyl CoA must be shuttled back into the cytoplasm. The acetyl CoA combines with oxaloacetic acid to form citrate. The mitochondrial membrane is permeable to citrate. In the cytoplasm the citrate is converted back to acetyl CoA and oxaloacetic acid. The NADPH is from the hexosemonophosphate shunt discussed in Chapter 3.

Essential Fatty Acids

Recall that human cells cannot introduce additional double bonds beyond the Δ^9 site because they lack enzymes called Δ^{12} and Δ^{15} desaturases. That is why linoleic acid (18:2 $\Delta^{9,12}$) and α -linolenic acid (18:3 $\Delta^{9,12,15}$) are essential fatty acids. They can be acquired from plant sources because plant cells do have the desaturase enzymes. Once linoleic acid is acquired, longer, more highly unsaturated fatty acids can be formed from it by a combination of elongation and desaturation reactions. Figure 5.30 illustrates the elongation and desaturation of palmitate and linoleate. These elongation reactions produce fatty acids that are metabolized to biologically active compounds that play a significant physiological role, described in the next section.

Eicosanoids: Fatty Acid Derivatives of Physiological Significance

Linoleic acid and α -linolenic acid are essential because they act as precursors for some longer, more highly unsaturated fatty acids, which in turn are necessary to form cell membranes and as precursors of compounds called eicosanoids. Eicosanoids are fatty acids composed of 20 carbon atoms. They include the physiologically potent families of substances called **prostaglandins**, **thromboxanes**, and **leukotrienes**, all of which are formed from precursor fatty acids by the incorporation of oxygen atoms into the fatty acid chains. Reactions of this sort are often called **oxygenation reactions**, and the enzymes catalyzing the reactions are named oxygenases.

The most important fatty acid serving as a precursor for eicosanoid synthesis is arachidonate. Its oxygenation follows either of two major pathways:

- the “cyclic” pathway, which results in the formation of prostaglandins and thromboxanes
- the “linear” pathway, which produces leukotrienes

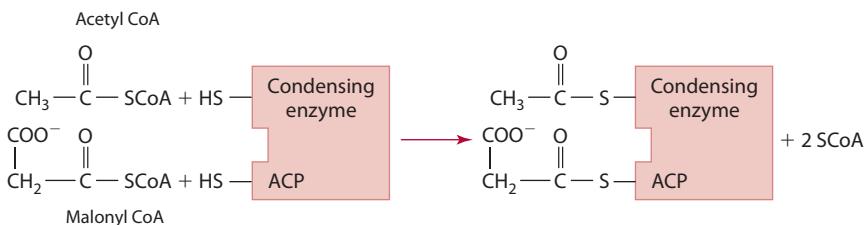


Figure 5.28 “Loading” of sulphydryl groups into the fatty acid synthase system.

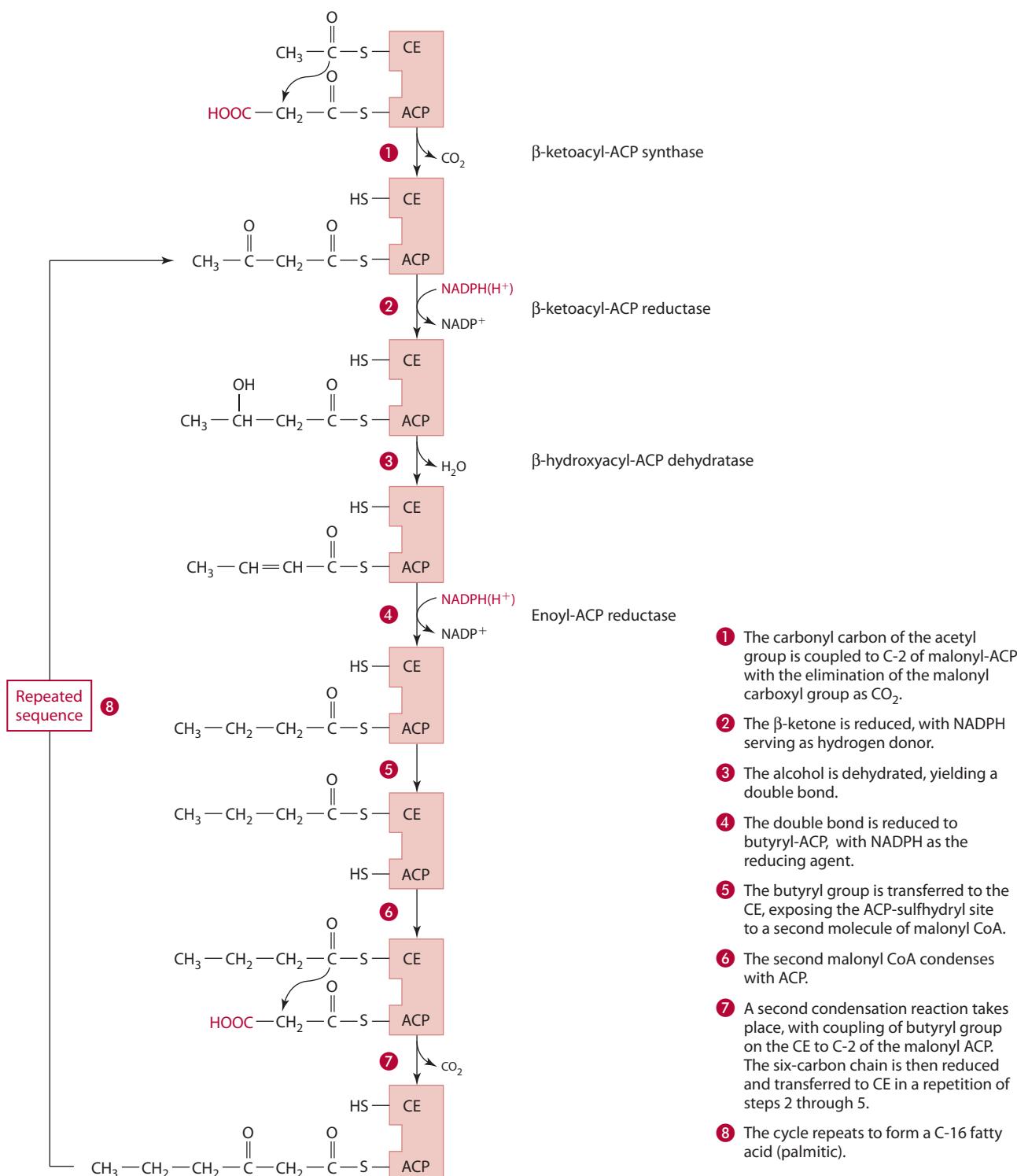


Figure 5.29 The steps in the synthesis of fatty acid. CE (condensing enzyme) and ACP (acyl carrier protein) are members of a complex of enzymes referred to as the fatty acid synthase system.

The featured enzyme in the cyclic pathway is prostaglandin endoperoxide synthase, sometimes called cyclo-oxygenase. It catalyzes the oxygenation of arachidonate together with the cyclization of an internal segment of the arachidonate chain, the hallmark structural feature of the prostaglandins

and thromboxanes. The enzyme that converts arachidonate to the leukotrienes in the linear pathway is lipoxygenase, and the pathway is often called the lipoxygenase pathway. Figure 5.31 is an overview of the reactions of the cyclic and linear pathways of arachidonic acid.

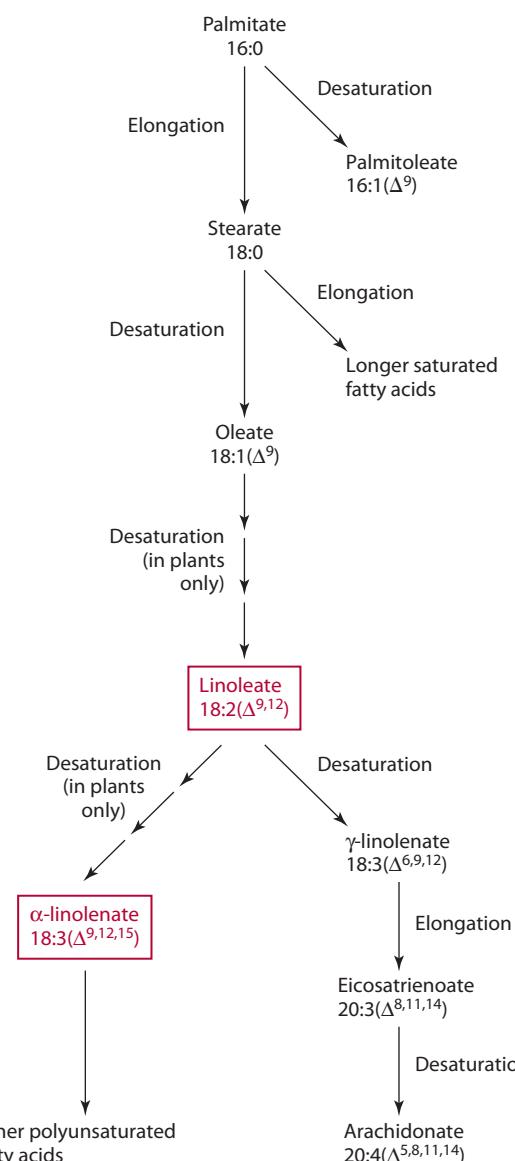


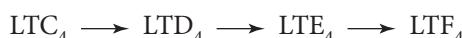
Figure 5.30 Routes of synthesis of other fatty acids from palmitate as precursor. Elongation and desaturation reactions allow palmitate to be converted into longer and more highly unsaturated fatty acids. Mammals cannot convert oleate into linoleate or α-linolenate. These fatty acids are therefore essential and must be acquired in the diet.

Prostaglandins (PG) are 20-carbon fatty acids that have a 5-carbon ring in common but display modest structural differences among themselves. As shown in Figure 5.31, they are designated PGD, PGE, PGF, PGI, PGG, and PGH; subscripts indicate the number of double bonds, with the “2” series being the most important. These compounds, along with the thromboxanes, exhibit a wide range of physiological actions, including the lowering of blood pressure, diuresis, blood platelet aggregation, effects on the immune and nervous systems as well as gastric secretions, and the stimulation of smooth muscle contraction, to name several. They are described as being “hormonelike” in function. However, hormones originate from a specific gland, and

their actions are the same for all their target cells, whereas prostaglandins are widely distributed in animal tissues but affect only the cells in which they are synthesized. They do appear to alter the actions of hormones, often through their modulation of cAMP levels and the intracellular flow of calcium ions.

Certain combinations of prostaglandins and thromboxanes may exhibit antagonistic effects. For example, prostacyclin (PGI_2) is a potent stimulator of adenylate cyclase and thereby acts as a platelet “antiaggregating” factor, because platelet aggregation is inhibited by cAMP. Opposing this action is thromboxane A_2 , which inhibits adenylate cyclase and consequently serves as a “proaggregating” force. Another example of opposing actions of the prostaglandins is the vasodilation of blood vessels by PGE_2 and their vasoconstriction by PGF_2 .

Certain prostaglandins produce a rise in body temperature (fever) and can cause inflammation and therefore pain. The anti-inflammatory and antipyretic (fever-reducing) activity of aspirin, acetaminophen, and indomethacin is attributable to their inhibitory effect on prostaglandin endoperoxide synthase (cyclo-oxygenase), which results in reduced prostaglandin and thromboxane synthesis. Cyclooxygenase (abbreviated as COX) is present in two isoforms. COX-1 carries out the normal physiological production of prostaglandins. The second isoform, COX-2, is induced within inflammatory cells and is responsible for the production of prostaglandins in inflammation. One class of anti-inflammatory drugs consists of COX-2 inhibitors. The 5-lipoxygenase pathway of leukotriene (LTC_4) formation from arachidonate is shown in Figure 5.30. Although not shown in the pathway, LTC_4 is further metabolized to other leukotrienes in the following order:



The structures of LTA_4 and LTC_4 are shown because they exemplify a leukotriene and a peptidoleukotriene, respectively. Note that LTC_4 is formed from LTA_4 by incorporation of the tripeptide glutathione (γ -glutamylcysteinylglycine). LTD_4 , LTE_4 , and LTF_4 are peptidoleukotrienes produced from LTC_4 by peptidase hydrolysis of bonds within the glutathione moiety. Like the prostaglandins, these substances share structural characteristics but are classified within the A, B, C, D, and E series according to their structural differences. As for prostaglandins, the subscript number represents the number of double bonds in the compound.

Leukotrienes have potent biological actions. Briefly, they contract respiratory, vascular, and intestinal smooth muscles. The effects on the respiratory system include constriction of bronchi and increased mucus secretion. These actions, which are known to be expressed through binding to specific receptors, have implicated the leukotrienes as mediators in asthma, immediate hypersensitivities, inflammatory reactions, and myocardial infarction. In fact, one of

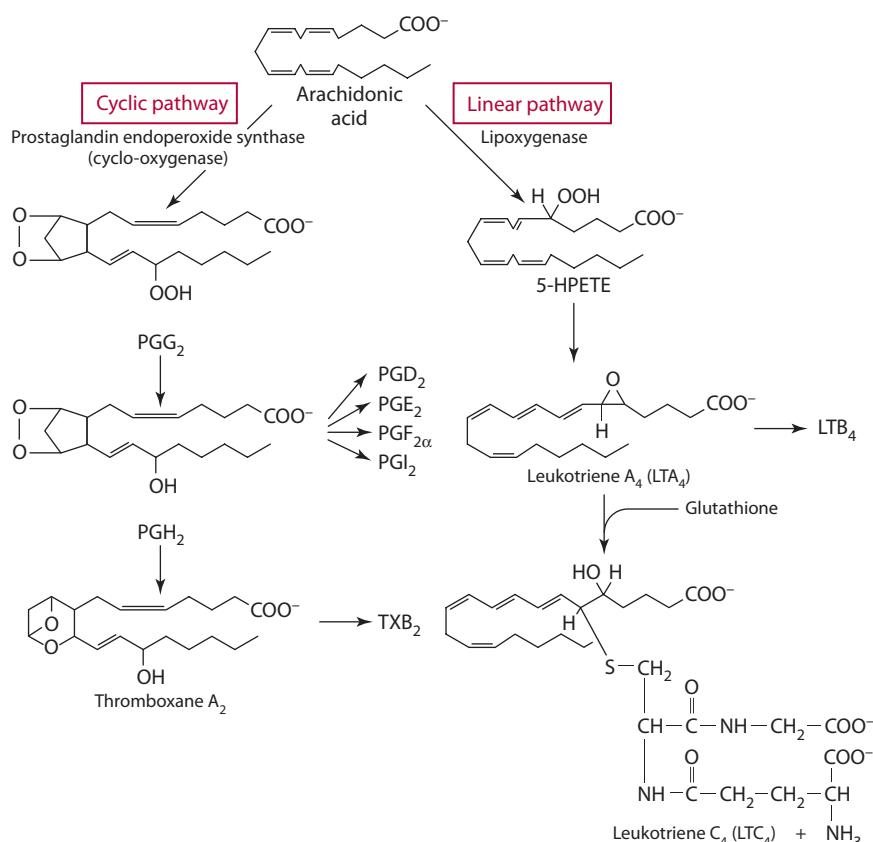


Figure 5.31 The formation of prostaglandins, thromboxanes, and leukotrienes from arachidonic acid via cyclo-oxygenase and lipoxygenase pathways. Abbreviations: PG, prostaglandin; TX, thromboxane; 5-HPETE, 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid.

the major chemical mediators of anaphylactic shock—the so-called slow-reacting substance of anaphylactic shock, or SRS-A—has been found to be a mixture of the peptidoleukotrienes LTC₄, LTD₄, and LTE₄. Anaphylactic shock is a life-threatening response to chemical substances, primarily histamine, that are released as a result of a severe allergic reaction.

A necessity for eicosanoid formation is the availability of an appropriate amount of free (unesterified) arachidonate. Cellular concentration of the free fatty acid is not adequate and therefore must be released from membrane glycerophosphatides by a specific hydrolytic enzyme called phospholipase A₂. Structural features of glycerophosphatides were reviewed in the section on phospholipids. The most important glycerophosphatides acting as sources of arachidonate in cells are phosphatidylcholine and phosphatidylinositol. When present in these structures, arachidonate normally occupies the *sn*-2 position. Recall that the *sn*-2 position of phospholipids normally is occupied by a polyunsaturated fatty acid.

The release of arachidonate from membrane glycerophosphatides, for eicosanoid synthesis, is influenced by stimuli. These stimuli are of two main types, physiological (specific) and pathological (nonspecific). Physiological stimulation, a natural occurrence, is brought about by stimulatory compounds such as epinephrine, angiotensin

II, and antigen-antibody complexes. Pathological stimuli, which result in a more generalized release of all fatty acids from the *sn*-2 position, include mechanical damage, ischemia, and membrane-active venoms. Table 5.5 lists the precursors, sites of synthesis, and physiological effects of a few major eicosanoid groups.

In addition to the prostaglandins made from arachidonic acid, an n-6 fatty acid, another series is made from eicosapentaenoic acid, an n-3 fatty acid. A few of the major compounds in this series are shown in Table 5.5. The physiological action of these two series of hormonelike substances is too extensive to be covered here. In general, the two series have opposing actions.

Essential Fatty Acid in Development

Both ω-6 and ω-3 essential fatty acids are found in both adults and infants. They both are metabolized by the same series of desaturases and elongases to longer-chain polyunsaturated fatty acids as described above. Deficiency symptoms for the ω-6 series have been identified in adults and include poor growth and scaly skin lesions. Deficiency symptoms for the ω-3 series include neurological and visual abnormalities. Human infants have been observed to have similar neurological abnormalities when maintained on a regimen that was lacking in 18:3ω3. Human milk contains more of the essential fatty acids (but the

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level varies) than most infant formulas do. They also contain the elongated derivatives. There is evidence that ω -3 essential fatty acids are necessary for neural tissue and retinal photoreceptor membranes. Both term and preterm infants can convert ω -3 essential fatty acids to the long-chain polyunsaturated fatty acids, but whether they can convert them at an adequate rate to meet their needs is unclear. ω -3 essential fatty acid deficiency appears to be more common among preterm infants than with term infants. Infant formulas containing 22:6 ω 3 and 20:4 ω 3 are now available [30].

Impact of Diet on Fatty Acid Synthesis

The rate of fatty acid synthesis can be influenced by diet. Diets high in simple carbohydrates and low in fats induce a set of lipogenic enzymes in the liver. This induction is exerted through the process of transcription, leading to elevated levels of the mRNA for the enzymes. The transcriptional response is triggered by an increase in glucose metabolism, and the triggering substance, though not positively identified, is thought to be glucose 6-phosphate [31]. Other studies have confirmed that a very low fat–high sugar diet causes an increase in fatty acid synthesis and in palmitate-rich, linoleate-poor VLDL triacylglycerols. Furthermore, the effect may be reduced if starch is substituted for the sugar, possibly owing to the slower absorption of starch glucose and a lower postprandial insulin response [32].

SYNTHESIS OF TRIACYLGLYCEROLS (TRIGLYCERIDES)

The biosyntheses of triacylglycerols and glycerophosphatides share common precursors and are considered together in this section. The precursors are CoA-activated fatty acids and glycerol 3-phosphate, the latter of which is produced either from the reduction of dihydroxyacetone phosphate or from the phosphorylation of glycerol. These and subsequent reactions of the pathways are shown in Figure 5.32. The figure depicts two pathways for lecithin synthesis from diacylglycerol. The de novo pathway of lecithin synthesis is the major route. However, the importance of the salvage pathway increases when a deficiency of the essential amino acid methionine exists.

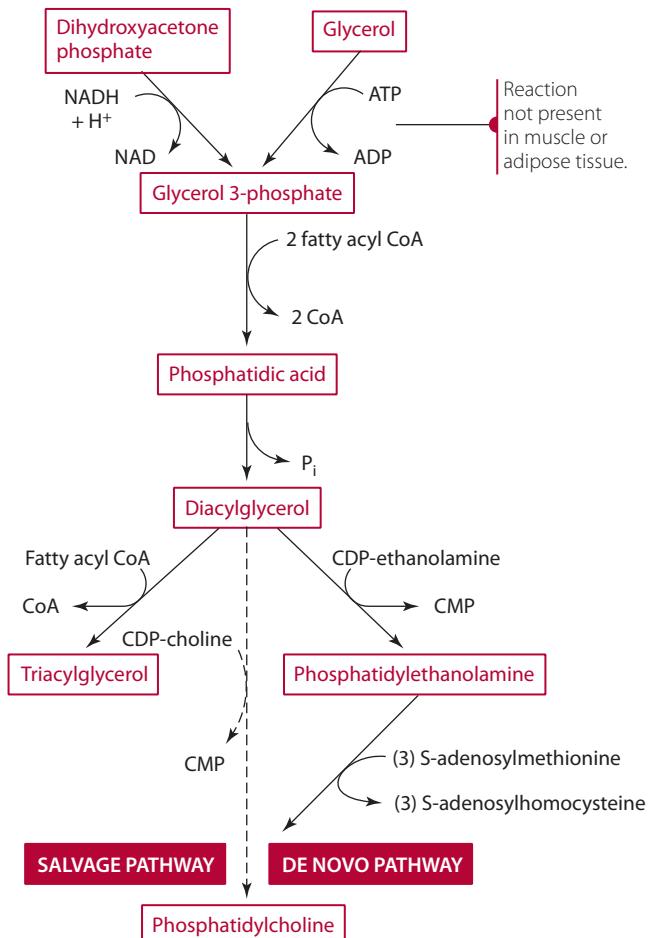


Figure 5.32 A schematic summary of the synthesis of triacylglycerols and lecithin showing that precursors are shared. In lecithin formation, three moles of activated methionine (S-adenosylmethionine) introduce three methyl groups in the de novo pathway, and choline is introduced as CDP (cytidine diphosphate) choline in the so-called salvage pathway.

SYNTHESIS OF CHOLESTEROL

Nearly all tissues in the body are capable of synthesizing cholesterol from acetyl CoA. The liver accounts for about 20% of endogenous cholesterol. Among the extrahepatic tissues, which are responsible for the remaining 80% of synthesized cholesterol, the intestine is probably the most active. The cholesterol production rate, which includes both absorbed cholesterol and endogenously synthesized cholesterol,

approximates 1 g/day. Compare this with the recommended dietary intake of about 300 mg/day. The average cholesterol intake is considered to be about 600 mg/day, only about half of which is absorbed. Endogenous synthesis therefore accounts for more than two-thirds of the daily total.

At least 26 steps are known to be involved in the formation of cholesterol from acetyl CoA. The individual steps are not provided here, but the synthesis of cholesterol can be thought of as occurring in three stages:

1. a cytoplasmic sequence by which 3-hydroxy-3-methylglutaryl CoA (HMG CoA) is formed from 3 mol of acetyl CoA
2. the conversion of HMG CoA to squalene, including the important rate-limiting step of cholesterol synthesis, in which HMG CoA is reduced to mevalonic acid by HMG CoA reductase
3. the formation of cholesterol from squalene

As total body cholesterol increases, the rate of synthesis tends to decrease. This is known to be caused by a negative

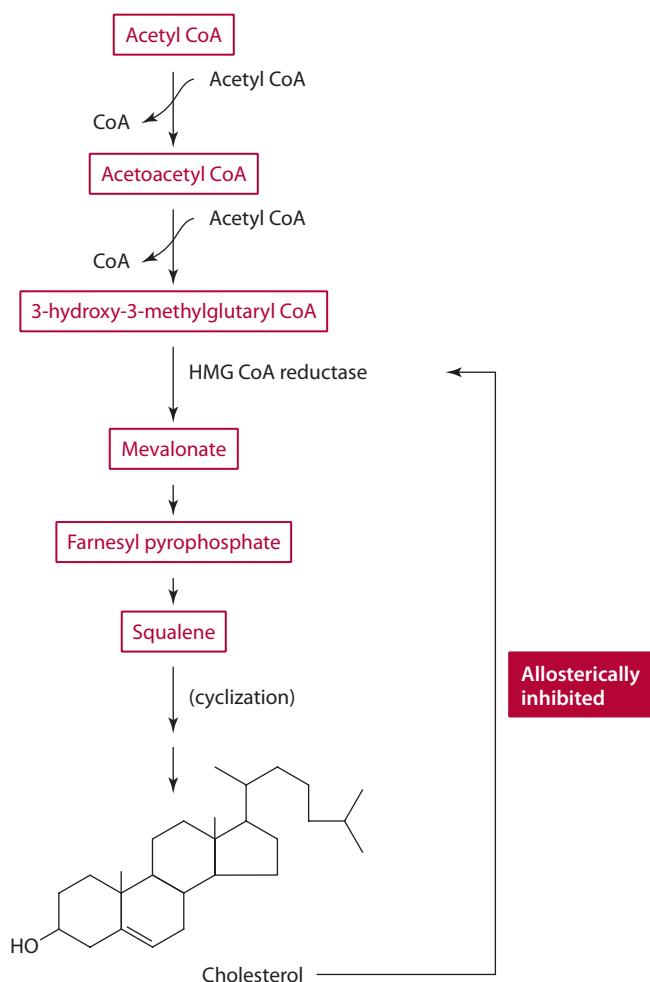


Figure 5.33 An overview of the pathway of cholesterol biosynthesis in the hepatocyte indicating the negative regulatory effect of cholesterol on the HMG CoA reductase reaction.

feedback regulation of the HMG CoA reductase reaction. This suppression of cholesterol synthesis by dietary cholesterol seems to be unique to the liver and is not much evident in other tissues. The effect of feedback control of biosynthesis depends to a great extent on the amount of cholesterol absorbed. The suppression is not sufficient to prevent an increase in the total body pool of cholesterol when dietary intake is high. A brief scheme of hepatic cholesterologenesis and its regulation is shown in Figure 5.33.

Regulation of Lipid Metabolism

The regulation of fatty acid oxidation is closely linked to carbohydrate status. Fatty acids formed in the cytoplasm of liver cells can either be converted into triacylglycerols and phospholipids or be transported via carnitine into the mitochondrion for oxidation. The enzyme carnitine acyl transferase I, which catalyzes the transfer of fatty acyl groups to carnitine (Figure 5.22), is specifically inhibited by malonyl CoA. Recall that malonyl CoA is the first intermediate in the synthesis of fatty acids. Therefore, it is logical that an increase in the concentration of malonyl CoA would promote fatty acid synthesis while inhibiting fatty acid oxidation. Malonyl CoA concentration increases whenever a person is well supplied with carbohydrate. Excess glucose that cannot be oxidized through the glycolytic pathway or stored as glycogen is converted to triacylglycerols for storage, using the available malonyl CoA. Therefore, glucose-rich cells do not actively oxidize fatty acids for energy. Instead, a switch to lipogenesis is stimulated, accomplished in part by inhibition of the entry of fatty acids into the mitochondrion.

Blood glucose levels can affect lipolysis and fatty acid oxidation by other mechanisms as well. Hyperglycemia triggers the release of insulin, which promotes glucose transport into the adipose cell and therefore promotes lipogenesis. Insulin also exerts a pronounced antilipolytic effect. Hypoglycemia, on the other hand, results in a reduced intracellular supply of glucose, thereby suppressing lipogenesis. Furthermore, the low level of insulin accompanying the hypoglycemic state would favor lipolysis, with a flow of free fatty acids into the bloodstream. Low glucose levels also stimulate the rate of fatty acid oxidation in the manner described in the section dealing with the ketone bodies. In this case, accelerated oxidation of fatty acids follows the reduction in TCA cycle activity, which in turn results from inadequate oxaloacetate availability.

The key enzyme for the mobilization of fat is hormone-sensitive triacylglycerol lipase, found in adipose tissue cells. Lipolysis is stimulated by hormones such as epinephrine and norepinephrine, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), glucagon,

growth hormone, and thyroxine. Insulin, as mentioned earlier, antagonizes the effects of these hormones by inhibiting the lipase activity.

A very important allosteric enzyme involved in the regulation of fatty acid biosynthesis is acetyl CoA carboxylase, which forms malonyl CoA from acetyl CoA (Figure 5.27). The enzyme, which functions in the cytoplasm, is positively stimulated by citrate. In the absence of this modulator, the enzyme is barely active. Recall that citrate is part of the shuttle for moving acetyl CoA from the mitochondria (a major site of production) to the cytoplasm where fatty acids are synthesized. Citrate is continuously produced in the mitochondrion as a TCA cycle intermediate, but its concentration in the cytoplasm is normally low. When mitochondrial citrate concentration increases, it can escape to the cytoplasm, because the mitochondrial membrane is permeable to citrate. In the cytoplasm it acts as a positive allosteric signal to acetyl CoA carboxylase, thereby increasing the rate of formation of malonyl CoA, which results in lipogenesis. The result of citrate accumulation is that excess acetyl CoA is diverted to fatty acid synthesis and away from TCA cycle activity.

Acetyl CoA carboxylase can be modulated negatively by palmitoyl CoA, which is the end product of fatty acid synthesis. This situation would most likely arise when free fatty acid concentrations increase as a result of insufficient glycerophosphate, with which fatty acids must combine to form triacylglycerols. Deficient glycerophosphate levels would likely stem from inadequate carbohydrate availability. In such a situation, regulation would logically favor fatty acid oxidation rather than synthesis.

A great deal of interest in serum cholesterol levels has been generated because of their correlation to the risk of cardiovascular disease. The regulation of cholesterol homeostasis is associated with its effect on LDL receptor concentration and on the activity of regulatory enzymes such as acyl CoA:cholesterol acyltransferase (ACAT) and hydroxymethylglutaryl CoA (HMG CoA) reductase. Cholesterol's feedback suppression of HMG CoA reductase has been discussed and is shown in Figure 5.33. The combination of increasing ACAT activity (the conversion of free cholesterol to cholestryl esters) and decreasing the amount of LDL receptors reduces the accumulation of cholesterol in vascular endothelial and smooth muscle cells. Figure 5.19 illustrates these mechanisms of control.

Brown Fat Thermogenesis

Brown adipose tissue obtains its name from its high degree of vascularity and the abundant mitochondria present in the adipocytes. Recall that the mitochondria are pigmented, owing to the cytochromes and perhaps other oxidative pigments associated with electron transport. Not only do

brown fat cells contain larger numbers of mitochondria than white fat cells do, but the mitochondria also are structurally different, to promote **thermogenesis** (heat production) at the expense of producing ATP.

Brown fat mitochondria have special H⁺ pores in their inner membranes, formed by an integral protein called thermogenin, or the uncoupling protein (UCP). UCP is a translocator of protons, which allows the external H⁺ pumped out by electron transport to flow back into the mitochondria rather than through the F₀F₁ ATP synthase site of phosphorylation. Remember that it is the H⁺ gradient that causes the conformational changes that result in the phosphorylation of ADP to produce ATP. Figure 5.34 illustrates how the proposed mechanism of brown fat thermogenesis relates to the chemiosmotic theory of oxidative phosphorylation. Protons within the mitochondrial matrix are pumped outside the inner membrane by the electron transport energy. Then the downhill flow of protons through the F₀F₁ aggregate channels provides the energy for ADP phosphorylation. Membrane pores of brown fat mitochondria allow the cycling of protons, which lowers the proton concentration in the inner membrane space and results in heat generation rather than ATP production. This cycling appears to be regulated by the 32,000-dalton UCP.

Two types of external stimuli trigger thermogenesis: (1) ingestion of food and (2) prolonged exposure to cold temperature. Both of these events stimulate the tissue via sympathetic innervation via the hormone norepinephrine. The sympathetic signal has a stimulatory and hypertrophic effect on brown adipose tissue. This effect enhances expression of the UCP in the inner membrane of the mitochondrion and accelerates synthesis of lipoprotein lipase and glucose transporters to make more fatty acids and glucose available [33]. An additional pathway for enhanced UCP activity, independent of the sympathetic nervous system, involves retinoic acid stimulation. A 27-base-pair sequence in the UCP gene has been identified as the retinoic acid receptor. Upon binding to its receptor in brown fat adipocytes, retinoic acid stimulates the transcriptional activity of the gene and therefore the synthesis of UCP [34].

Figure 5.34 includes the pathways for the stimulation of UCP expression. The higher UCP concentration allows a greater proton flux into the matrix, which in turn encourages greater electron transport activity in answer to the reduced proton pressure in the intermembrane space. Enhancement of lipoprotein lipase and glucose transporters provides fuel (fatty acids and glucose, respectively) to meet the higher metabolic demand. The end result of this stimulation is that the phosphorylation of ADP by electron transport in the mitochondria of the brown fat cells becomes "uncoupled," which leads to less ATP formation but considerably more heat production. As ATP production diminishes, the dynamics of the catabolic breakdown and the anabolic biosynthesis of stored nutrients will shift to catabolism in an effort to replenish the ATP.

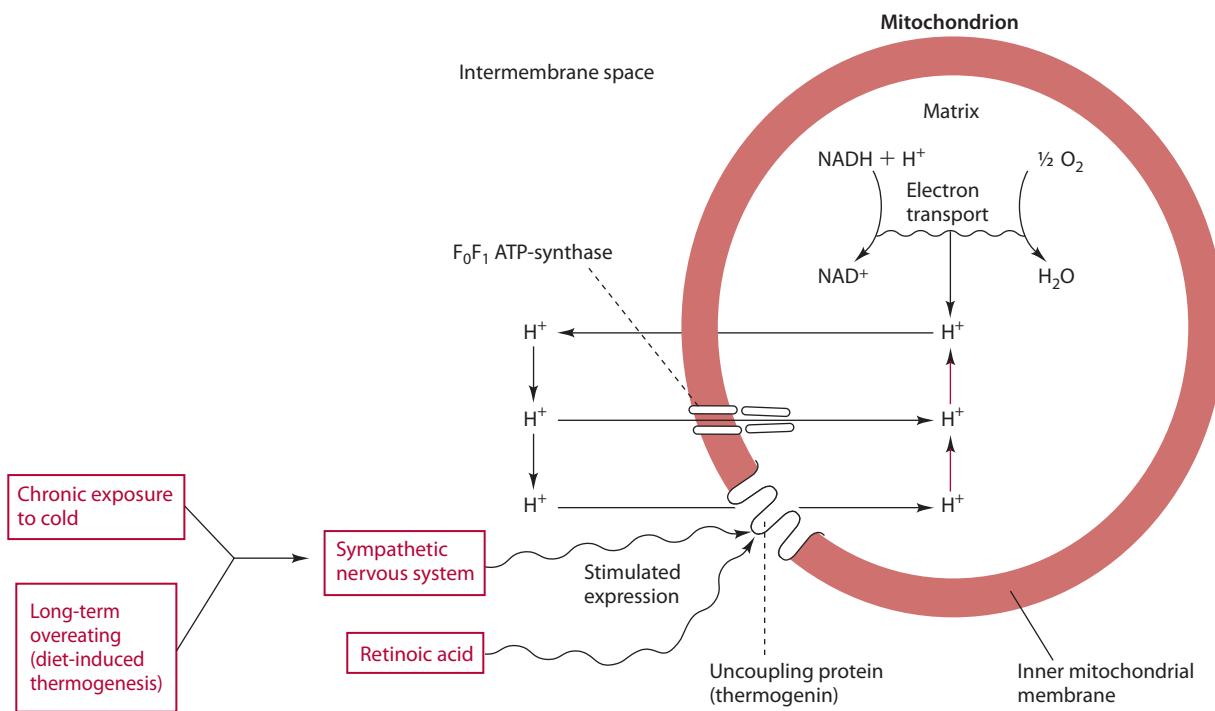


Figure 5.34 The stimulatory effect of some mediators on brown adipocyte thermogenesis. Retinoic acid has been shown to act independently of the sympathetic nervous system. Enhanced activity of the uncoupling protein, which translocates protons from the intermembrane space of the mitochondrion to the matrix, shifts the energy released from the proton flow toward thermogenesis and away from ATP synthesis.

Theoretically, then, weight reduction should accompany a higher activity of brown fat, and, indeed, a possible link between obesity and deficient brown fat cell function has been researched. For example, evidence indicates that thermogenesis is defective in instances of obesity. However, direct evidence that the defect resides in brown adipose tissue is tenuous. Studies using thermographic skin measurements have shown defective thermogenesis in the brown fat depots of obese subjects who had received catecholamine hormone, but such measurements are imprecise because of the insulation provided by the thick, subcutaneous adipose tissue of obese people. In fact, some researchers are of the opinion that a simple, single test to assess appropriate brown fat function in obese subjects is lacking. Despite this shortcoming, the demonstration of the catecholamine stimulation of brown fat thermogenesis deserves further investigation. It raises the tempting possibility that brown adipose tissue may relate to human obesity both in a causal way and as a focus for diet therapy.

achieve, however, because fat enhances palatability, which is a major driving force in food selection. The synthetic compound Olestra was developed as a fat replacement to impart palatability to a food without the consequences of high-calorie fat. Orlistat has a different mode of action, interfering with the digestion and absorption of natural dietary fat. Olestra is a mixture of hexa-, hepta-, and octaesters of sucrose with long-chain fatty acids. It imparts a taste essentially indistinguishable from that of fat, yet it cannot be hydrolyzed by the pancreatic lipases and therefore has no caloric value. Technically, it can replace fat in a wide variety of foods and can be used to reduce fat-derived calories in cooked, baked, or fried foods. Because undigested food entering the bowel can cause intestinal irritability, Olestra was viewed unfavorably as a probable contributor to such discomfort. In fact, some early reports linked Olestra with colonic irritability, with accompanying gas and diarrhea. Although this issue remains under debate, most well-designed studies have exonerated Olestra in this regard. In one such investigation, substituting up to 30 g of Olestra in a meal of 45 g fat had no effect on gastric, small intestinal, or colonic transit time [35]. This finding has been corroborated by numerous other studies. Furthermore, the overall safety of Olestra is supported by some positive findings, not only from the standpoint of its harmlessness to the gastrointestinal tract but also regarding areas such as metabolism, absorption, mutagenicity, carcinogenicity, and nutrition [36].

Therapeutic Inhibition of Fat Absorption: Olestra and Orlistat

Because of the association between dietary fat, obesity, and cardiovascular disease, reducing fat intake is a logical nutritional recourse. This goal has been difficult to

Still under study, however, is whether reducing fat energy intake might trigger an appetite increase as an energy-compensatory mechanism which would increase the intake of carbohydrate. Pronounced compensatory biobehavioral responses may accompany severe reductions in energy from fat, making adherence to the low-fat diet difficult to maintain [37]. The number of products containing Olestra appearing on the shelves of supermarkets appears to be declining, which suggests a lack of acceptance by the consumer.

Orlistat

Orlistat is a semisynthetic derivative of lipstatin, which is a naturally occurring, potent inhibitor of gastric and pancreatic lipase. Recall that although hydrolytic products of triacylglycerols—monoacylglycerols and free fatty acids—are absorbed across the intestinal epithelium, intact triacylglycerols are not. The rationale for Orlistat use, therefore, is that by restricting the hydrolysis of triacylglycerols, the compound will sharply reduce absorption of triacylglycerols.

Orlistat acts by binding covalently to the serine residue of the active site of gastric and pancreatic lipase and shows little or no inhibitory activity against α -amylase, trypsin, chymotrypsin, or the phospholipases. Dose response curves show that a plateau of inhibition of dietary fat absorption occurs at high levels of the drug (>400 mg/day), corresponding to approximately 35% inhibition. At therapeutic doses (300–400 mg/day) taken in conjunction with a well-balanced, mildly hypocaloric diet, Orlistat inhibits fat absorption as much as approximately 30%, contributing to a caloric deficit of about 200 calories. The drug is available in a prescription-only form and an over-the-counter form (a lower dose). It does not appear to cause significant gastrointestinal disturbances or to affect gastric emptying and acidity, gallbladder motility, bile composition, biliary stone formation, or systemic electrolyte balance [38,39].

Ethyl Alcohol: Metabolism and Biochemical Impact

Ethyl alcohol is neither a carbohydrate nor a lipid. Empirically, however, ethanol's structure ($\text{CH}_3\text{—CH}_2\text{—OH}$) most closely resembles a carbohydrate. Its metabolism most closely resembles fatty acid catabolism, and we have chosen to review it in this chapter for several reasons. First, it is a common dietary component, being consumed in the form of alcoholic beverages such as beer, wines, and distilled spirits. Second, the pathways that oxidize ethyl alcohol also oxidize (or detoxify) other exogenous substances in the body. Although ethanol is not a “natural” nutrient, it does have caloric value (its calories are “empty,” that is, devoid of beneficial nutrients). Each gram of ethanol

yields 7 kcal, and ethanol may account for up to 10% of the total energy intake of moderate consumers and up to 50% for alcoholics. Because of its widespread consumption and relatively high caloric potency, it commands attention in a nutrition textbook.

Ethanol is readily absorbed through the entire gastrointestinal tract. It is transported unaltered in the bloodstream and then oxidatively degraded in tissues, primarily the liver, first to acetaldehyde and then to acetate. In tissues peripheral to the liver, as well as in the liver itself, the acetate subsequently is converted to acetyl CoA and oxidized via the TCA cycle. At least three enzyme systems are capable of ethanol oxidation:

- alcohol dehydrogenase (ADH)
- the microsomal ethanol oxidizing system (MEOS; also known as the cytochrome P-450 system)
- catalase, in the presence of hydrogen peroxide

Of these, the catalase- H_2O_2 system is the least active, probably accounting for <2% of *in vivo* ethanol oxidation. Therefore, we will not discuss the catalase system further. Nearly all ingested ethanol is oxidized by hepatic (and, to some extent, gastric) alcohol dehydrogenase and hepatic microsomal cytochrome P-450 systems.

THE ALCOHOL DEHYDROGENASE (ADH) PATHWAY

ADH is a soluble enzyme functioning in the cytoplasm of liver cells. It is an ordinary NAD^+ -requiring dehydrogenase and is known to be able to oxidize ethanol to acetaldehyde. The NADH formed by the reaction can be oxidized by mitochondrial electron transport by way of the NADH shuttle systems (see Chapter 3) thereby giving rise to ATP formation by oxidative phosphorylation. The K_m of alcohol dehydrogenase for ethanol is approximately 1 mM, or about 5 mg/dL. (K_m is reviewed in Chapter 1, in the section dealing with enzymes.) This means that at cellular concentration of ethanol, ADH is functioning at half its maximum velocity. At concentrations three or four times the K_m , the enzyme is saturated with the ethanol substrate and is catalyzing at its maximum rate. Concentrations of ethanol in the cell more than four times the K_m level cannot be oxidized by ADH.

Because ethanol is an exogenous dietary ingredient, there is no “normal” concentration of ethanol in the cells or the bloodstream. The so-called toxic level of blood ethanol, however, is considered to be in the range of 50 to 100 mg/dL and is defined by its pharmacological actions. The high lipid solubility of ethanol allows it to enter cells passively with ease. If its cellular concentration were to reach a level even one-third or one-fourth that in blood, ADH would be saturated by the substrate and would be functioning at its maximum velocity. The excess, or “spillover,”

ethanol then must be metabolized by alternate systems, the most important of which is the microsomal ethanol oxidizing system (MEOS), described next. Another factor forcing a shift to the microsomal metabolizing system is a depletion of NAD⁺, brought about by the high level of activity of ADH. The microsomal system does not require NAD⁺ for its oxidative reactions.

Alcohol dehydrogenase activity is also found in gastric mucosal cells. Interestingly, a significant gender difference appears to be present in the level of its activity in these cells. Young (premenopausal) females develop higher blood alcohol levels than male counterparts with equal consumption and consequently display a lower tolerance for alcohol and are at greater risk of toxic effects in the liver. The lower level of alcohol dehydrogenase activity in the female gastric mucosa is believed to account for this observation [40,41].

THE MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS)

Despite its name, the microsomal ethanol oxidizing system (MEOS) is able to oxidize a wide variety of compounds in addition to ethanol, including fatty acids, aromatic hydrocarbons, steroids, and barbiturate drugs. The oxidation occurs through a system of electron transport, similar to the mitochondrial electron transport system described in detail in Chapter 3. Because the MEOS is microsomal and is associated with the smooth endoplasmic reticulum, it is sometimes referred to as the microsomal electron transport system. Another distinction of the system is its requirement for a special cytochrome called cytochrome P-450, which acts as an intermediate electron carrier. Cytochrome P-450 is not a single compound but rather exists as a family of structurally related cytochromes, the members of which share the property of absorbing light that has a wavelength of 450 nm.

Ethanol oxidation by the MEOS is linked to the simultaneous oxidation of NADPH by molecular oxygen. Because two substrates therefore are oxidized concurrently, the enzymes involved in the oxidations are commonly called mixed-function oxidases. One oxygen atom of the oxygen molecule is used to oxidize NADPH to NADP⁺, and the second oxidizes the ethanol substrate to acetaldehyde. Both oxygen atoms are reduced to H₂O, and therefore two

H₂O molecules are formed in the reactions. Microsomal electron transport of the MEOS is shown in Figure 5.35. Acting as carriers of electrons from NADPH to oxygen are FAD, FMN, and a cytochrome P-450 system.

An important feature of the MEOS is that certain of its enzymes, including the cytochrome P-450 units, are inducible by ethanol. This characteristic means that ethanol, particularly at higher concentrations, can induce the synthesis of these substances. The result is that the hepatocytes can metabolize ethanol much more effectively, thereby establishing a state of metabolic tolerance. Compared with a normal (nondrinking or light-drinking) subject, an individual in a state of metabolic tolerance to ethanol can ingest larger quantities of the substance before showing the effects of intoxication. When enzyme induction occurs, however, it can also accelerate the metabolism of other substances metabolized by the microsomal system. In other words, tolerance to ethanol induced by heavy drinking can render a person tolerant to other substances in addition to ethanol.

ALCOHOLISM: BIOCHEMICAL AND METABOLIC ALTERATIONS

Excessive consumption of ethanol can lead to alcoholism, defined by the National Council on Alcoholism as consumption that is capable of producing pathological changes. Alcoholism is a serious socioeconomic and health problem, exemplified by the fact that in the United States alcohol-related liver disease has been reported as the sixth leading cause of death [42]. The well-known consequences of alcoholism—fatty liver, hepatic disease (cirrhosis), lactic acidosis, and metabolic tolerance—can be explained by the manner in which ethanol is metabolized. Basically, the consequences of excessive alcohol intake are explainable by metabolic effects of (1) acetaldehyde toxicity, (2) elevated NADH:NAD⁺ ratio, (3) metabolic competition, and (4) induced metabolic tolerance.

A comprehensive review of the association of nutritional and biochemical alteration with alcoholism is available [43].

Acetaldehyde Toxicity

Both the ADH and the MEOS routes of ethanol oxidation produce acetaldehyde, which is believed to exert

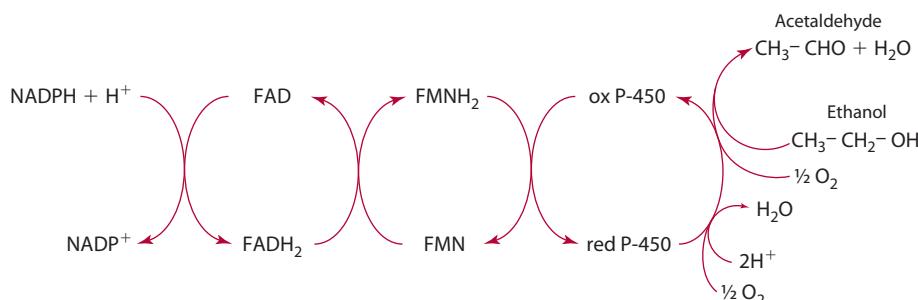


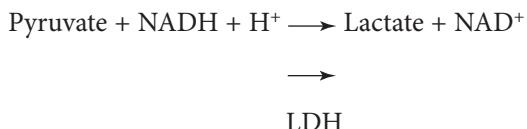
Figure 5.35 The microsomal ethanol oxidizing system (MEOS). Both ethanol and NADPH are oxidized by molecular oxygen in this electron transfer scheme. Because two substrates are oxidized, enzymes involved in the oxidation are referred to as mixed-function oxidases.

direct adverse effects on metabolic systems. For example, acetaldehyde is able to attach covalently to proteins, forming protein adducts. Should the adduct involve an enzyme, the activity of that enzyme could be impaired. Acetaldehyde has also been shown to impede the formation of microtubules in liver cells and to cause the development of perivenular fibrosis, either of which is believed to initiate the events leading to cirrhosis. These and other possible adverse effects of acetaldehyde are reviewed by Lieber [44].

Alcoholic cirrhosis was once thought to be caused by malnutrition, because the drinker satisfied his or her caloric needs with the “empty” calories of alcohol at the expense of a nutritionally balanced diet. In view of the effect of high levels of acetaldehyde on hepatocyte structure and function, however, chronic overindulgence is now known to cause cirrhosis even when nutritional deficiency is absent and the alcohol is co-ingested with an enriched diet.

High NADH:NAD⁺ Ratio

The oxidation of ethanol increases the concentration of NADH at the expense of NAD⁺, therefore elevating the NADH:NAD⁺ ratio. This process occurs because both ADH and acetaldehyde dehydrogenase use NAD⁺ as cosubstrate. NADH is an important regulator of certain dehydrogenase reactions. The rise in concentration of NADH represents an overproduction of reducing equivalents, which in turn acts as a signal for a metabolic shift toward reduction—namely, hydrogenation. Such a shift can account for fatty liver (through the anabolic activity producing fatty acids) and lactic acidemia (high blood-lactate levels), which often accompany alcoholism, by increasing the reduction of pyruvate to lactic acid. For example, lactic acidemia can be attributed in part to the direct effect of NADH in shifting the lactate dehydrogenase (LDH) reaction toward the formation of lactate. The reaction, which follows, is driven to the right by the high concentration of NADH:



Lipids accumulate in most tissues in which ethanol is metabolized, resulting in fatty liver, fatty myocardium, fatty renal tubules, and so on. The mechanism appears to involve both increased lipid synthesis and decreased lipid removal and can be explained in part by the increased NADH:NAD⁺ ratio. As NADH accumulates, it slows dehydrogenase reactions of the TCA cycle, such as the isocitrate dehydrogenase and α -ketoglutarate dehydrogenase reactions, thereby slowing the overall activity of the cycle. This results in an accumulation of citrate, which positively regulates acetyl CoA carboxylase. Acetyl

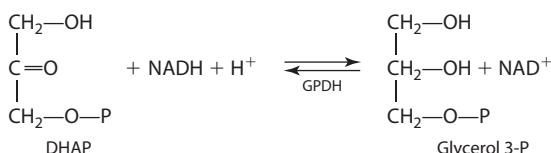


Figure 5.36 The reaction of dihydroxyacetone (DHAP) to glycerol 3-P, producing NAD⁺ from NADH.

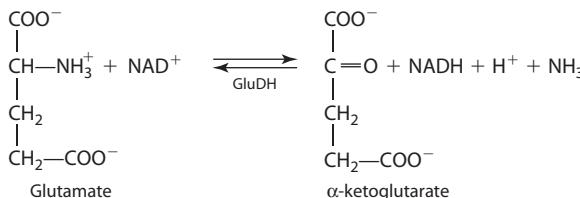


Figure 5.37 The reversible reaction of glutamate and NAD⁺ forming α -ketoglutarate, NADH, and ammonia, catalyzed by glutamate dehydrogenase (GluDH).

CoA carboxylase converts acetyl CoA into malonyl CoA by the attachment of a carboxyl group. It is the key regulatory enzyme for the synthesis of fatty acids from acetyl CoA. The high NADH:NAD⁺ ratio therefore directs metabolism away from TCA cycle oxidation and toward fatty acid synthesis.

Also contributing to the lipogenic effect of alcoholism is the effect of NADH on the glycerophosphate dehydrogenase (GPDH) reaction. The reaction, shown in Figure 5.36, favors the reduction of dihydroxyacetone phosphate (DHAP) to glycerol 3-phosphate if NADH concentration is high. Glycerol 3-phosphate provides the glycerol component in the synthesis of triacylglycerols. Therefore, a high NADH:NAD⁺ ratio stimulates the synthesis of both the fatty acids and the glycerol components of triacylglycerols, contributing to the cellular fat accumulation that develops in alcoholism.

The glutamate dehydrogenase (GluDH) reaction (Figure 5.37) also is affected by a rise in NADH concentration, resulting in impaired gluconeogenesis. The GluDH reaction is extremely important in gluconeogenesis because of the role it plays in the conversion of amino acids to their carbon skeletons by transamination and in the release of their amino groups as NH₃. A shift in the reaction toward glutamate because of the elevated NADH depletes the availability of α -ketoglutarate, which is the major acceptor of amino groups in the transamination of amino acids.

Substrate Competition

A well-established nutritional problem associated with excessive alcohol metabolism is a deficiency of vitamin A. Two aspects of ethanol interference with normal metabolism probably can account for this problem. One is the effect of ethanol on retinol dehydrogenase, the

cytoplasmic enzyme that converts retinol to retinal. Retinal is required for the synthesis of photopigments used in vision. Retinol dehydrogenase is thought to be identical to ADH, and therefore ethanol competitively inhibits the hepatic conversion of retinol to retinal. In addition to this substrate competition effect, ethanol may interfere with retinol metabolism through induced metabolic tolerance.

Induced Metabolic Tolerance

As explained earlier, ethanol can induce enzymes of the MEOS, causing an increased rate of metabolism of substrates oxidized by this system. Retinol, like ethanol, spills over into the MEOS when ADH is saturated and NAD⁺ stores are low because of heavy ingestion of ethanol. Ethanol induction of retinol-metabolizing enzymes then can occur. The specific component of the MEOS known to be induced by heavy consumption of ethanol has been designated cytochrome P-450IIE1. Although induction accelerates the hepatic oxidation of retinol, the oxidation product is not retinal but other polar, inert products of oxidation. The hepatic depletion of retinol can therefore be attributed to its accelerated metabolism, which is secondary to ethanol induction of a metabolizing enzyme. In effect, the alcoholic subject becomes tolerant to vitamin A, necessitating a higher dietary intake of the vitamin to maintain normal hepatocyte concentrations.

ALCOHOL IN MODERATION: THE BRIGHTER SIDE

Alcohol is a nutritional “Jekyll and Hyde,” and which face it flaunts is clearly a function of the extent to which it is consumed. We focused earlier on the effects of alcohol at high intake levels and the negative impact of alcoholism on metabolism and nutrition. Many studies, however, have suggested that alcohol consumed in moderation may have beneficial effects, particularly in its ability to improve plasma lipid profiles and reduce the risk of cardiovascular disease.

Ethanol is known to elevate the level of high-density lipoprotein (HDL) in serum and to lower the amount of serum lipoprotein a [45,46]. Both effects favor a decrease in cardiovascular disease risk. This is because HDL protects against the deposition of arterial fatty plaque (atherogenesis), whereas high levels of lipoprotein(a) appear to promote it. The effect of these and other lipoproteins on atherogenesis were discussed earlier in this chapter. Other mechanisms for the apparent protective action of alcohol against atherogenesis have been suggested. A well-recognized component of the atherogenic process is the proliferation of smooth muscle cells underlying the endothelium of arterial walls. Studies have shown that alcohol may suppress the proliferation of smooth muscle cells, thereby slowing the atherogenic process [47].

SUMMARY

The hydrophobic character of lipids makes them unique among the major nutrients, requiring special handling in the body's aqueous milieu. Ingested fat must be finely dispersed in the intestinal lumen in order to present a sufficiently large surface area for enzymatic digestion to occur. In the bloodstream, reassembled lipid must be associated with proteins to ensure its solubility in that environment while undergoing transport. The major sites for the formation of lipoproteins are the intestine, which produces them from exogenously derived lipids, and the liver, which forms lipoproteins from endogenous lipids. Central to the processes of fat transport and storage is adipose tissue, which accumulates fat as triacylglycerol when the intake of energy-producing nutrients is greater than the body's caloric needs. When the energy demand so dictates, fatty acids are released from storage and transported to other tissues for oxidation. The mobilization follows the adipocyte's response to specific hormonal signals that stimulate the activity of the intracellular lipase.

Fatty acids are a rich source of energy. Their mitochondrial oxidation furnishes large amounts of acetyl CoA for TCA cycle catabolism, and in situations of low carbohydrate intake or use, as occurs in starvation or diabetes, the rate of fatty acid oxidation increases significantly with

concomitant acetyl CoA accumulation. This causes an increase in the level of the ketone bodies—organic acids that can be deleterious through their disturbance of acid-base balance but that also are beneficial as sources of fuel to tissues such as muscle and brain in periods of starvation.

Although the lipids are thought of first and foremost as energy sources, some can be identified with intriguing hormonelike functions ranging from blood pressure alteration and platelet aggregation to enhancement of immunological surveillance. These potent bioactive substances are the prostaglandins, thromboxanes, and leukotrienes, all of which are derived from the fatty acids, arachidonate, and certain other long-chain PUFAs.

Dietary lipid has been implicated in atherogenesis, the process leading to development of the degenerative cardiovascular disease called atherosclerosis. Major considerations in preventing and controlling this disease have been the concentration of cholesterol in the blood serum and the relative hypocholesterolemic or hypercholesterolemic effect of certain diets. Saturated fatty acids having medium-length chains, along with unsaturated *trans* fatty acids, are alleged to be hypercholesterolemic, whereas mono- and polyunsaturated *cis* fatty acids tend to lower serum cholesterol.

Fats can be synthesized by cytoplasmic enzyme systems when energy production by carbohydrate is adequate. The synthesis begins with simple precursors such as acetyl CoA and can be triggered by hormonal signals or by elevated levels of citrate, which acts as a regulatory substance. Blood glucose concentration also acts as a sensitive regulator of lipogenesis, which is stimulated when a hyperglycemic state exists.

Ethanol is catabolized ultimately to acetyl CoA, furnishing energy through the TCA cycle oxidation. The nutritional complexities of alcohol abuse were discussed.

Protein metabolism will be surveyed in Chapter 6. There you will learn that amino acids, like carbohydrates and lipids, can furnish energy through their oxidation or

can be metabolically converted into other substances of biochemical importance. Once again, remember that the metabolic pathways of the energy nutrients are linked through common metabolites. Gluconeogenesis, discussed in Chapter 3, illustrates this integration through the formation of carbohydrate from the glycerol moiety of lipids and certain amino acids. Chapter 6 will demonstrate how protein can be converted into fat through common intermediates. The expression “A rose is a rose is a rose” applies quite appropriately to intermediary metabolites. For example, if acetyl CoA is the substrate for an enzyme, the enzyme will convert it with no regard as to whether it originated through carbohydrate, lipid, or protein metabolism.

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Suggested Readings

Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986; 232:34–47.

This is an excellent step-by-step review of the research resulting in the delineation of the LDL receptor, its mechanisms of cholesterol homeostasis, and the therapeutic implications of these mechanisms.

Budowski P. Ω -3 fatty acids in health and disease. In: Bourne GH, ed. *World Review of Nutrition and Dietetics* 1988; 57:214–74.

The structural aspects, sources, and antithrombotic actions of the Ω -3 fatty acids are thoroughly reviewed, along with the eicosanoids and their multiplicity of physiological functions.

Web Sites

www.nal.usda.gov

This takes you to the National Agriculture Library and from there you can link to the U.S. Government Food and Nutrition Information Center

www.amhrt.org

American Heart Association

www.eatright.org

American Dietetic Association

The Role of Lipids and Lipoproteins in Atherogenesis

Atherogenesis is a degenerative systemic process involving arteries. If unchecked, the process becomes symptomatic, as vascular damage and impeded blood flow develop. The resulting disease state is called atherosclerosis. Although the disease is generally associated with old age, lesions begin to develop many years before symptoms become evident. The innermost layer of the arterial wall, which is in direct contact with the flowing blood, is called the intima. The intima consists of a layer of endothelial cells that act as a barrier to blood-borne cells and other substances. Underlying the intima is the media, consisting of layers of smooth muscle cells that make up the muscular component of the arterial wall. The atherogenic process is believed to begin in the endothelium, with subsequent or concurrent involvement of the medial smooth muscle cells.

The mechanism of atherogenesis is complex and not yet fully understood. However, two major components appear to be implicated:

- cells of the immune system, primarily monocytes and macrophages that are phagocytic cells, and T lymphocytes
- lipids and lipoproteins, most importantly LDL, oxidized or otherwise modified

These components function together in the disease process.

It has been known for some time that a high level of circulating cholesterol is a major risk factor for cardiovascular disease. More recently, however, convincing experimental evidence has implicated LDL more specifically as the prime contributor to the process. LDL is the major transporter of cholesterol in the serum. The concentration of circulating LDL is controlled by two factors:

- Its rate of formation from VLDL (see this chapter). Normally, about one-third of VLDL is converted to LDL, and two-thirds is removed by the liver by apoE receptors.
- Its fractional clearance rate. LDL is removed by LDL receptors, primarily (75%) in the liver but also in other tissues. A small amount is removed by cellular endocytosis, the so-called scavenger process.

Initiation of the atherogenic process may begin in response to some form of endothelial cell injury. This injury could result from mechanical stress such as hypertension or a high level of oxidized LDL, known to be toxic to endothelial cells. An increased adherence of monocytes and T lymphocytes to the affected area follows, along with infiltration of platelets. These cells are believed to be activated as a result of concurrent penetration of the endothelium by LDL. The following events, also listed in Table 1, then occur, although not necessarily in the order listed:

- Growth factors released from platelets stimulate the proliferation of smooth muscle cells in the arterial media. The smooth muscle cells accumulate lipid presented to them as LDL, transforming them into lipid-laden foam cells.
- Monocytes (or macrophages) also take up LDL particles, becoming foam cells. This uptake of LDL at the arterial wall is the scavenger pathway of LDL removal. It works independently of the LDL receptor.
- Macrophages, stimulated by the uptake of LDL, release additional growth factors and chemotactic factors (cytokines) that attract more macrophages to the site, creating more foam cells.

Most of the lipid in foam cells is cholesterol and cholestryl esters. As the foam cells proliferate, their lipid contents accumulate in the form of fatty streaks. These ultimately enlarge, occluding, to a matter of degree, the arterial lumen, which can become narrowed to the extent that blood flow is compromised.

LDL modified by oxidation is a stronger contributor to atherogenesis than is native LDL. Uptake of oxidized LDL by macrophages is much more rapid than uptake of native LDL. In fact, cultured macrophages do not take up native LDL *in vitro*. Also, oxidized LDL has a considerably stronger proliferative effect on smooth muscle cells, and it is more toxic to endothelial cells [1]. It has been proposed that oxidized LDL may play a central role in atherogenesis in at least three additional ways [2]:

- It acts as a chemoattractant for the blood-borne monocytes to enter the subendothelial space.
- It causes the transformation of monocytes into macrophages.
- It causes the trapping of macrophages in the endothelial spaces by inhibiting their motility.

The oxidizing power in the cells involved has not been identified, but metal ions such as Cu²⁺ and Fe³⁺, superoxide radicals, and heme-containing compounds have been suggested. Oxidation results in peroxidation of double bonds in the lipid moiety of the LDL particle. Cholesterol and cholestryl esters can be converted into 7-keto derivatives, and unsaturated fatty acids are oxidatively fragmented into shorter-chain aldehydes. The toxicity of 7-ketocholesterol is believed to induce apoptosis in smooth muscle cells, a process associated with the release of oxidizing species that can contribute further to lipid peroxidation [3]. It has been suggested that the shorter-chain aldehydes resulting from peroxidation of unsaturated fatty acids may attach covalently to the apoprotein B-100 component of the particle through lysine side chains. In its chemically modified form, but not in its unaltered form, the protein is recognized by receptors on scavenger cells such as macrophages. Therefore, uptake of LDL, and consequently foam cell production, is accelerated [2].

An overview of atherosclerosis published in a lay journal provides an understandable and well-illustrated look at current understanding of the development of atherosclerosis [4]. Evidence for the participation of oxidized LDL in the atherogenic process is certainly convincing. Although the benefit of supplementation with antioxidants such as vitamin E to slow the process, and therefore reduce the risk of cardiovascular disease, has yet to be determined, the rationale for doing so

Table 1 Proposed Events in the Process of Atherogenesis

Event
1 Native low-density lipoprotein (LDL) penetrates the arterial intima.
2 In the intima the polyunsaturated fatty acid esterified to the cholesterol in LDL is oxidized.
3 Oxidized LDL attracts macrophages.
4 The macrophages become lipid-engorged foam cells.
5 The oxidized LDL is cytotoxic to the endothelial cell creating an injury that attracts platelets.
6 Platelets release platelet-derived growth factor that stimulates the proliferation of the smooth muscle cells of the intima.
7 The smooth muscle cells take up oxidized LDL by endocytosis and become foam cells.
8 The macrophages, activated by the phagocytosis of oxidized LDL, release macrophage chemotaxins, which attracts additional macrophages, which perpetuate the process.
9 Eventually, the proliferation of foam cells and smooth muscle cells form a plaque, which enlarges enough to narrow the lumen of the artery, restricting blood flow.

is sound. The role of dietary intervention in reducing oxidized LDL and its atherogenic effects has been reviewed [5].

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Web Sites

- www.cspinet.org
Center for Science in the Public Interest
www.amhrt.org
American Heart Association

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6

Protein

Functional Categories

Catalysts Transporters
Messengers Buffers
Structural Elements Fluid Balancers
Immunoprotectors Other Roles

Protein Structure and Organization

Primary Structure Tertiary Structure
Secondary Structure Quaternary Structure

Amino Acid Classification

Structure Polarity
Net Electrical Charge Essentiality

Sources of Protein

Digestion and Absorption

Protein Digestion
Intestinal Brush Border Membrane Amino Acid and Peptide Absorption

Intestinal Basolateral Membrane Transport of Amino Acids
Intestinal Cell Amino Acid Use
Amino Acid Absorption into Extraintestinal Tissues

Amino Acid Metabolism

Synthesis of Plasma Proteins, Nitrogen-Containing Nonprotein Compounds, and Purine and Pyrimidine Bases

Protein Synthesis Overview

Amino Acid Catabolism Overview
Hepatic Catabolism and Uses of Aromatic Amino Acids
Hepatic Catabolism and Uses of Sulfur (S)-Containing Amino Acids
Hepatic Catabolism and Uses of the Branched-Chain Amino Acids
Hepatic Catabolism and Uses of Other Amino Acids

Amino Acids Not Taken Up by the Liver: Plasma Amino Acids and Amino Acid Pool(s)

Interorgan "Flow" of Amino Acids and Organ-Specific Metabolism

Glutamine and the Liver, Kidneys, and Intestine
Alanine and the Liver and Muscle
Skeletal Muscle
Kidneys
Brain and Accessory Tissues

Protein Turnover: Synthesis and Catabolism of Tissue Proteins

Cellular Protein Degradation Systems

Changes in Body Mass with Age

Protein Quality and Protein Intake

Evaluation of Protein Quality
Protein Information on Food Labels
Recommended Protein and Amino Acid Intakes
Protein Deficiency/Malnutrition

PERSPECTIVE

Protein Turnover: Starvation Compared with "Stress"

The importance of protein in nutrition and health cannot be overemphasized. It is quite appropriate that the Greek word chosen as a name for this nutrient is *proteos*, meaning “primary” or “taking first place.” Proteins are found throughout the body, with over 40% of body protein found in skeletal muscle, over 25% found in body organs, and the rest found mostly in the skin and blood. Proteins are essential nutritionally because of their constituent amino acids, which the body must have in order to synthesize its own variety of proteins and nitrogen-containing molecules that make life possible. Each body protein is unique in the characteristics and sequence pattern of the amino acids that comprise its structure.

This review of protein focuses on the functional roles of various body proteins. It also examines how protein is digested and amino acids are subsequently absorbed and metabolized. Protein needs associated with tissue protein synthesis and catabolism are addressed in this chapter, as are changes in body protein with aging. Lastly, the chapter reviews recommended intakes of protein, protein quality, and protein deficiency.

Functional Categories

The molecular architecture and activity of living cells depend largely on proteins, which make up over half of the solid content of cells and which show great variability in size, shape, and physical properties. Their physiological roles also are quite variable, and because of this variability, categorizing proteins according to their functions can be helpful in the study of human metabolism. This type of categorization demonstrates the body’s dependence on properly functioning proteins and provides a basis for understanding the significance of protein structure.

CATALYSTS

Enzymes are protein molecules (generally designated by the suffix *-ase*) that act as catalysts: they change the rate of reactions occurring in the body. Enzymes are found both intracellularly and extracellularly, such as in the blood. Enzymes are frequently classified according to the type of reactions that they catalyze. For example:

- Hydrolases cleave compounds.
- Isomerases transfer atoms within a molecule.
- Ligases (synthases) join compounds.

- Oxidoreductases transfer electrons.
- Transferases move functional groups.

Enzymes are necessary for sustaining life. They are constructed so that they combine selectively with other molecules (called substrates) in the cell. The active site on the enzyme (a small region usually in a crevice of the enzyme) is where the enzyme and substrate bind and the product is generated. Some enzymes, however, require a cofactor or coenzyme to carry out the reaction. Minerals such as zinc, iron, and copper function as cofactors for some enzymes. *Metalloprotein* is the name typically used for proteins to which minerals are complexed. Some, but not all, metalloproteins have enzymatic activity. B vitamins serve as coenzymes for many enzymes. *Flavoprotein* is the term generally used for protein enzymes bound to flavin mononucleotide (FMN) or flavin adenine nucleotide (FAD), coenzyme forms of the B vitamin riboflavin. Most human physiological processes require enzymes to promote chemical changes that could not otherwise occur. Some examples of physiological processes that depend on enzyme function include digestion, energy production, blood coagulation, and excitation and contraction of neuromuscular tissue.

MESSENGERS

Some proteins are hormones. **Hormones** act as chemical messengers. They are synthesized and secreted by endocrine tissue (glands) and transported in the blood to target tissues or organs, where they bind to protein receptors. Hormones generally regulate metabolic processes, for example, by promoting enzyme synthesis or affecting enzyme activity.

Whereas some hormones are derived from cholesterol and classified as steroid hormones, others are derived from one or more amino acids. The amino acid tyrosine, for example, is used along with the mineral iodine to synthesize the thyroid hormones. Tyrosine is also used to synthesize the catecholamines, including dopamine, norepinephrine, and epinephrine. The hormone melatonin is derived in the brain from the amino acid tryptophan. Other hormones are made up of one or more polypeptide chains. Insulin, for example, consists of two polypeptide chains linked by a disulfide bridge. Glucagon, parathyroid hormone, and calcitonin each consist of a single polypeptide chain. Many other peptide hormones, such as adrenocorticotrophic hormone (ACTH), somatotropin (growth hormone), and vasopressin (also known as antidiuretic hormone, ADH), have important roles in human metabolism and nutrition. These hormones are discussed throughout this chapter and the book.

STRUCTURAL ELEMENTS

Several proteins have structural roles in the body. Some of these proteins include:

- contractile proteins
- fibrous proteins
- globular proteins

The two main contractile proteins, actin and myosin, are found in cardiac, skeletal, and smooth muscles. Skeletal muscle is found throughout the body and is under voluntary control. Contraction is calcium-induced and involves not only actin and myosin, but also troponin and tropomyosin. Smooth muscle is found in many tissues including, for example, blood vessels, the lungs, the uterus, and the gastrointestinal tract. Smooth muscle is under involuntary control and contracts in response to calcium-induced phosphorylation of the structural protein myosin.

Fibrous proteins, which tend to be somewhat linear in shape, include collagen, elastin, and keratin and are found in bone, teeth, skin, tendons, cartilage, blood vessels, hair, and nails. Collagen is a group of well-studied proteins. Each type of collagen is made of three polypeptide (tropocollagen) chains that are cross-linked for strength. These chains, rather than forming specific secondary structures (α -helices or β -pleated sheets, discussed in the protein structure section), form a helical arrangement. The amino acid composition of the chains is rich in the amino acids glycine and proline. In addition, collagen contains two hydroxylated amino acids—hydroxylysine and hydroxyproline—that are not found in other proteins. Collagen polypeptides are also attached to carbohydrate chains and thus are considered to be glycoproteins.

Other structural proteins, such as elastin, are associated with proteoglycans. Both glycoproteins and proteoglycans are conjugated proteins and are discussed further in the section “Other Roles.”

Globular proteins are named for their spherical shape. Although they vary to some degree, depending on the exact protein, globular proteins generally contain multiple α -helices and β -pleated sheets (see the section “Protein Structure and Organization”). Some examples of globular proteins include myoglobin, calmodulin, and many enzymes.

IMMUNOPROTECTORS

Immunoprotection is provided to the body in part by a group of proteins called **immunoproteins**, also called immunoglobulins (Ig) or antibodies (Ab). These immunoproteins, of which there are five major classes (IgG, IgA, IgM, IgE, and IgD), are Y-shaped proteins made of four polypeptide chains (two small chains called light [L] chains and two large chains called heavy [H] chains). The immunoglobulins are produced by plasma cells derived from B-lymphocytes, a type of white blood cell. Immunoglobulins function by binding to antigens and inactivating them. Antigens typically consist of foreign

substances, such as bacteria or viruses that have entered the body. By complexing with antigens, immunoglobulins create immunoprotein-antigen complexes that can be recognized and destroyed through reactions with either complement proteins or cytokines. The complement proteins (approximately 20) are produced primarily in the liver and circulate in the blood and extracellular fluid. Cytokines are produced by white blood cells such as T-helper (CD4) cells and macrophages. In addition, white blood cells such as macrophages and neutrophils also destroy foreign antigens through the process of phagocytosis.

TRANSPORTERS

Transport proteins are a diverse group of proteins that combine with other substances (especially vitamins and minerals, but also other nutrients) to provide a means of carrying those substances in the blood, or into cells, or out of cells, or within cells. Transport proteins in cell membranes, for example, carry and thus regulate the flow of nutrients into and out of cells. Several types of transporters exist in cell membranes. Some transporters (called uniporters) carry only one substance across cell membranes; other transporters (called symporters) carry more than one substance. For example, many amino acid transporters in the intestinal cell's brush border membrane function as uniporters or symporters. Antiporters, another type of cell membrane protein transporter, function by exchanging one substance for another. For example, the Na^+ , K^+ -ATPase pump transports three sodium ions out of the cell in exchange for two potassium ions, which enter the cell. Proteins in cell membranes carry substances other than amino acids. For example, the protein hCtr transports copper into intestinal cells. Protein transporters also are found in the blood. The protein hemoglobin, found in red blood cells, transports oxygen and carbon dioxide. Some of the many other transport proteins that are found in blood and that are of particular importance include:

- albumin, which transports a variety of nutrients such as calcium, zinc, and vitamin B_6
- transthyretin (also called prealbumin), which complexes with another protein, retinol binding protein, to transport retinol (vitamin A)
- transferrin, an iron transport protein
- ceruloplasmin, a copper transport protein
- lipoproteins, which transport lipids in the blood

The cholesterol and triglyceride core of lipoproteins is surrounded by phospholipids and a protein "coat." The

proteins in the lipoproteins are actually a group of about 10 different apoproteins that enable the lipid to be transported in the blood and help direct the lipoproteins to target cells, where they are used by body tissues.

BUFFERS

Proteins, because of their constituent amino acids, can serve as a buffer in the body and thus help to regulate acid-base balance. A **buffer** is a compound that ameliorates a change in pH that would otherwise occur in response to the addition of alkali or acid to a solution. The pH of the blood and other body tissues must be maintained within an appropriate range. Blood pH ranges from about 7.35 to 7.45, whereas cellular pH levels are often more acidic. For example, the pH of red blood cells is about 7.2, and that of muscle cells is about 6.9. The H^+ concentration within cells is buffered by both the phosphate system and the amino acids in proteins. The protein hemoglobin, for example, functions as a buffer in red blood cells. In the plasma and extracellular fluid, proteins and the bicarbonate system serve as buffers. Amino acids act as acids or bases in aqueous solutions such as those in the body by releasing and accepting hydrogen ions, thereby contributing to the buffering capacity of proteins in the body. The buffering ability of proteins can be illustrated by the reaction $\text{H}^+ + \text{protein} \rightleftharpoons \text{Hprotein}$.

FLUID BALANCERS

In addition to acid-base balance, proteins (along with other factors) influence fluid balance. The presence of protein in the blood and in cells helps maintain fluid balance, or stated differently, helps attract water and contribute to osmotic pressure. Loss of or diminished concentrations of proteins, such as albumin, in the blood plasma results in a decrease in plasma osmotic pressure. When protein concentrations in the blood are less dense than normal, fluid "leaks" out of the blood and into interstitial spaces, causing swelling (edema). Restoring adequate protein in the blood (for example, by infusing albumin intravenously) promotes diffusion of water from the interstitial space back into the blood.

OTHER ROLES

Proteins carry out many additional roles. For example, in cell membranes, proteins function in cell adhesion, and some further serve to transmit signals into and out of the cell. Proteins also serve as receptors on cell membranes. Proteins can function in storage roles. For example, some minerals like copper, iron, and zinc are stored in body

tissues bound to proteins; these proteins are often called metalloproteins.

Many proteins in the body are known as conjugated proteins. Conjugated proteins are proteins that are joined to nonprotein components. These conjugated proteins have many diverse roles in the body. Glycoproteins, one type of conjugated protein, represent a huge group of proteins with multiple functions. For example, mucus, which is found in body secretions, is rich in glycoproteins. Mucus both lubricates and protects epithelial cells in the body. Glycoproteins serve structural roles in connective tissue such as collagen and elastin, and in bone matrix. Some of the body's hormones, such as thyrotropin, are glycoproteins. Further, many of the body's blood proteins (such as transthyretin) needed to help maintain fluid and acid-base balance are glycoproteins. **Glycoproteins** consist of a protein covalently bound to a carbohydrate component. The carbohydrate in glycoproteins generally includes short chains of glucose, galactose, mannose, fucose, N-acetyl-glucosamine, N-acetylgalactosamine, and acetylneuraminic (sialic) acid at the terminal end of the oligosaccharide chain. The carbohydrate portion of the glycoprotein can make up as much as 85% of the glycoprotein's weight. The carbohydrate component is bound typically through an N-glycosidic linkage with asparagine's amide group in its side chain or through an O-glycosidic linkage with the hydroxy group in serine's or threonine's side chain. Another group of proteins with multiple roles in the body

are the proteoglycans. **Proteoglycans** are macromolecules with proteins covalently conjugated by O-glycosidic or N-glycosylamine linkages to glycosaminoglycans (formerly called mucopolysaccharides). Glycosaminoglycans consist of long chains of repeating disaccharides and comprise up to 95% of the weight of the proteoglycan. Proteoglycans make up, in part, the extracellular matrix (ground substance) that surrounds many mammalian tissues or cells such as skin, bone, and cartilage. Examples of proteoglycans include hyaluronic acid, chondroitin sulfate, keratan sulfate, dermatan sulfate, and heparan sulfate.

Protein Structure and Organization

The functional role of protein is determined by its basic structure and organization. The primary, secondary, and tertiary structures of proteins illustrate three key levels of organization. Some proteins have an additional fourth level of organization, the quaternary structure.

PRIMARY STRUCTURE

The primary structure of a protein is the sequence of and strong covalent bonds among amino acids that occur as the polypeptide chain is synthesized on the ribosomes. The primary structure of a protein is shown in Figure 6.1.

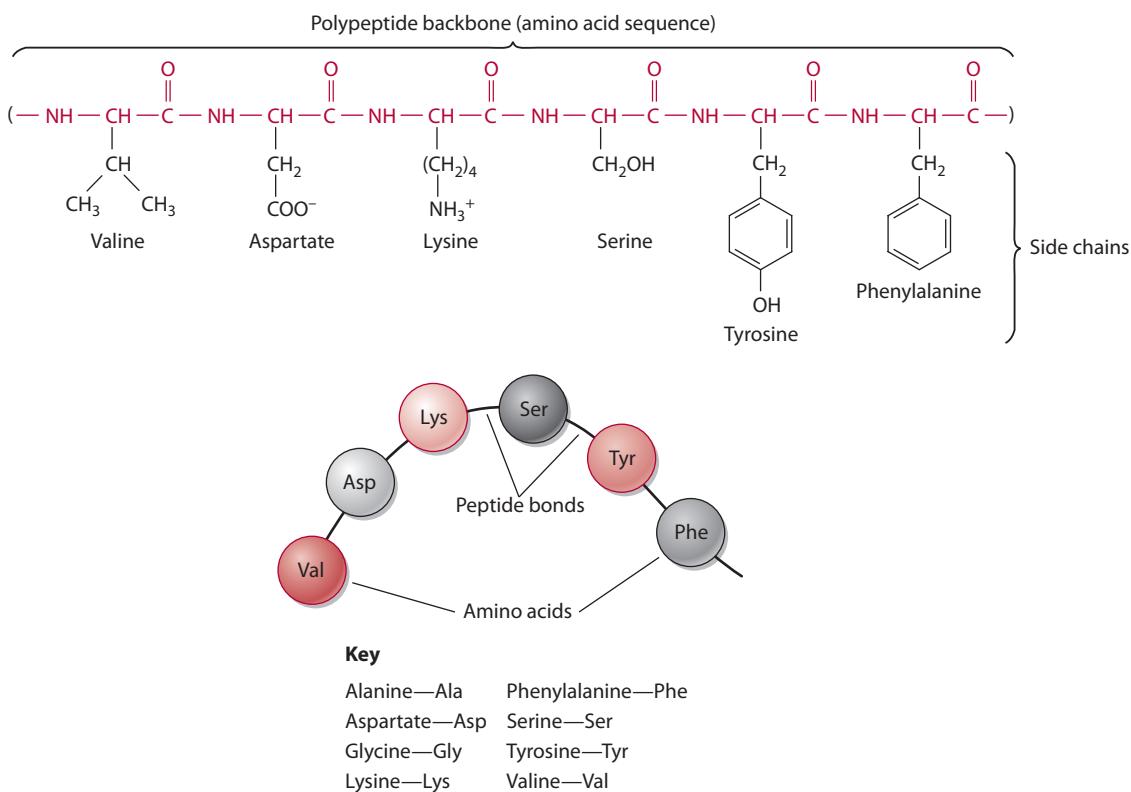


Figure 6.1 The primary structure of a protein.

The various amino acids that make up the polypeptide are labeled in sequence and represent the primary structure. The side chain of one amino acid differs from that of another amino acid, thus making each amino acid different. Polypeptide backbones do not differ between polypeptide chains.

The amino acid side chains in the polypeptide chain (or chains) that make up the total protein molecule account for the differences among proteins. Moreover, the side chains affect the way a protein coils and folds on itself, in effect helping to determine the final form (structure) of the protein molecule.

SECONDARY STRUCTURE

The secondary structure of a protein is achieved through weaker bonding, such as hydrogen bonding, than the

bonding that characterizes the primary structure. Hydrogen (H) bonds are weak electrical attractions that can occur between hydrogen atoms and negatively charged atoms such as oxygen or nitrogen. Weak repeating linkages between nearby amino acids account for this second level of protein organization.

One type of secondary structure of proteins is the α -helix, a cylindrical shape formed by a coiling of the polypeptide chain on itself, with interactions (H bonds) between the hydrogen atom attached to the amide nitrogen and the carbonyl oxygen atom occurring at every fourth peptide linkage (Figure 6.2a). The side chains of the amino acids in the α -helix structure extend outward. Varying degrees of the α -helix appear in widely divergent proteins, depending on their function. In those regions where it occurs, the α -helix provides some rigidity to that portion of the molecule.

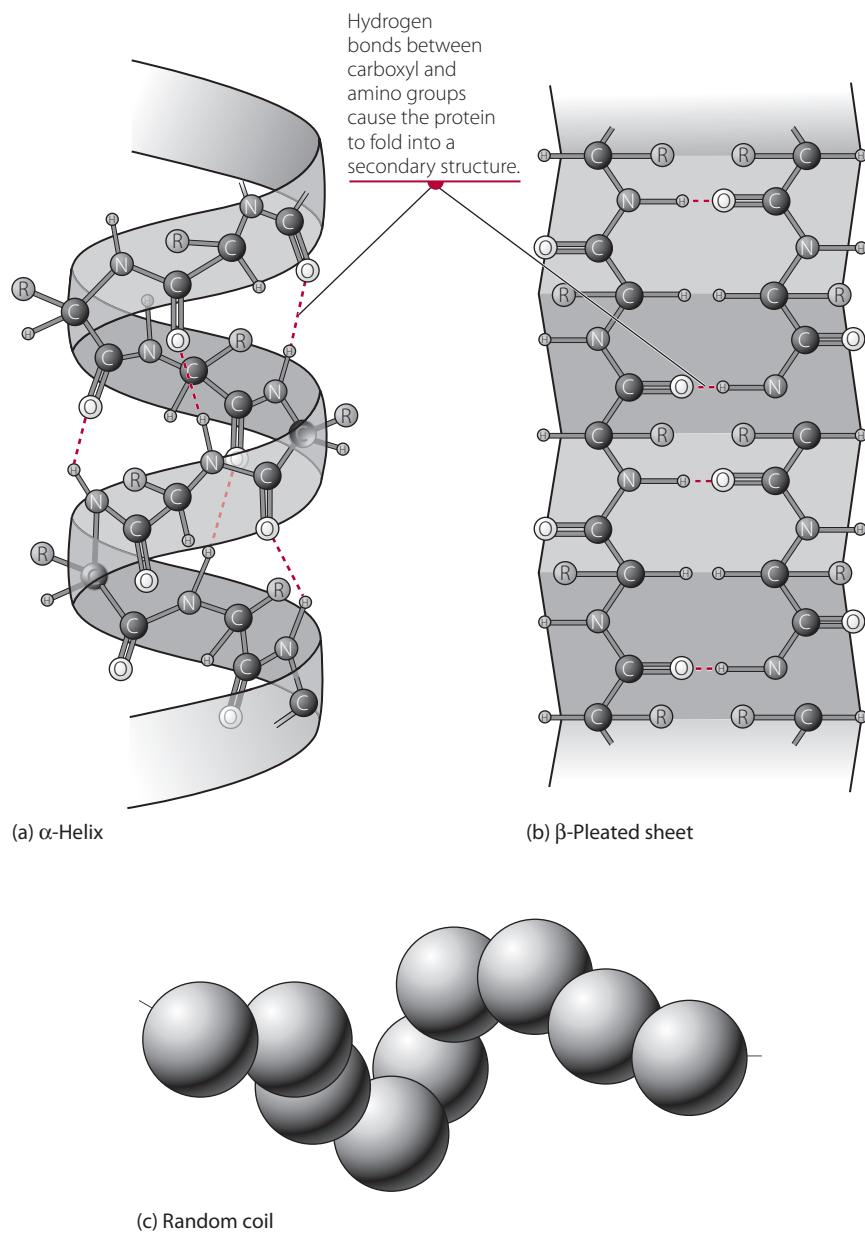


Figure 6.2 Secondary structure of proteins.

Another type of secondary protein structure is the β -conformation, or β -pleated sheet. In this structure, the polypeptide chain is fully stretched out, with the side chains positioned either up or down. The stretched polypeptide can fold back on itself with its segments packed together, as shown in Figure 6.2b. Both this structure and the α -helix are quite stable and provide strength and rigidity to proteins. These two secondary structures, α -helix and β -pleated sheet, are particularly abundant in proteins with structural roles, such as collagen, elastin, and keratin. Collagen, for example, is a triple helix composed of three long polypeptide α -chains. Each of the long polypeptide chains is “twisted” and covalently cross-linked within and between the triple helix units. The structure is tough and rodlike.

The random coil is the third type of secondary structure (Figure 6.2c). Little stability exists in this structure because of the presence of certain amino acids whose side chains interfere with one another.

TERTIARY STRUCTURE

The third level of organization in proteins is the tertiary structure: the way a protein folds in a three-dimensional space. This structure results from interactions among amino acid residues or side chains that are located at fairly close or considerable linear distances from each other along the peptide chain. These interactions can produce a linear, globular, or spherical structure, depending on the interactions. Interactions contributing to this third level of organization include:

- clustering of hydrophobic amino acids toward the center of the protein
- electrostatic attraction (also called ionic attraction) of oppositely charged amino acid residues, such as lysine (+1) and glutamate (-1)
- strong covalent bonding (involving electron sharing) between cysteine residues where the —SH groups are oxidized to form disulfide bridges (—S—S—)

Other, weaker attractions, such as hydrogen bonding among amino acid residues, usually also occur along the chain. Together, these interactions among the amino acid residues determine the protein's overall shape and, therefore, the particular function of the protein in the cell. Figure 6.3 shows an example of a tertiary structure of a protein and some of the interactions that create the tertiary structure.

QUATERNARY STRUCTURE

The final level of protein organization, quaternary structure, involves interactions between two or more polypeptide chains. Proteins with a quaternary structure most commonly are composed of either two or four polypeptide chains, and the aggregate formed is called an **oligomer**. The

polypeptide chains making up the oligomer, commonly termed subunits, are held together by hydrogen bonds and electrostatic salt bridges or attractions. Oligomeric proteins are particularly important in intracellular regulation, because the subunits can assume different spatial orientations relative to each other and, in so doing, change the properties of the oligomer. Hemoglobin (Figure 6.4), an oligomer with four subunits, illustrates this point. Each subunit of hemoglobin can bind one oxygen atom. Rather than acting independently, however, the subunits cooperate by conformational changes so as to enhance the affinity of hemoglobin for oxygen in the lungs and to increase its ability to unload oxygen in the peripheral tissues.

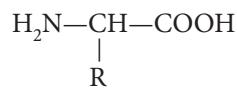
Other very important oligomers, such as regulatory enzymes, similarly undergo conformational changes on interaction with substrate molecules. In so doing, they enhance the formation of enzyme-substrate complexes whenever the concentration of substrate presented to the cell begins to increase. They also inhibit the formation of complexes when the substrate concentration falls to a low level.

Amino Acid Classification

Amino acids may be classified in a variety of ways, including by structure, net charge, polarity, and essentiality. This section addresses each of these four classifications.

STRUCTURE

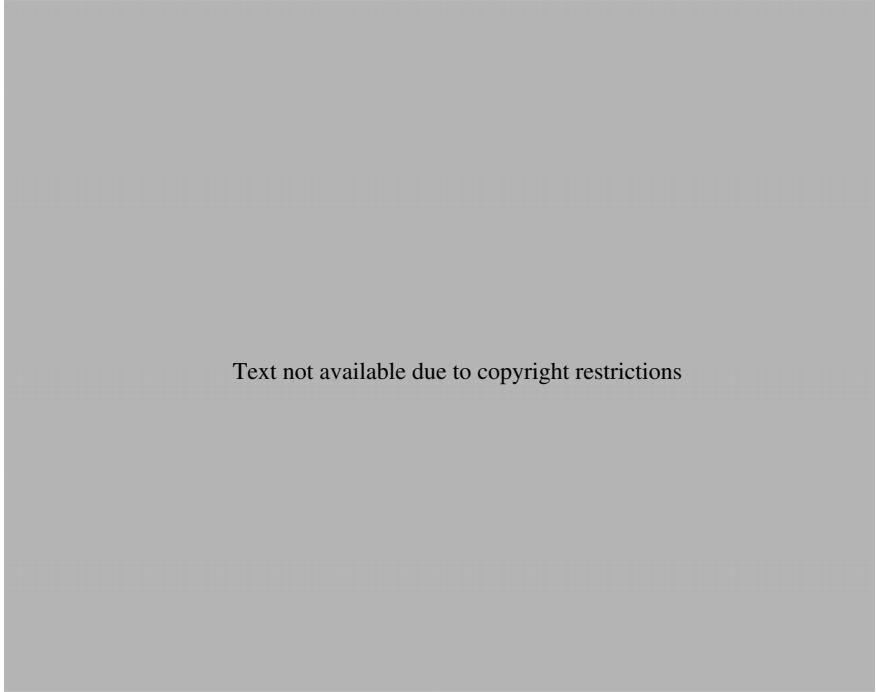
Structurally, all amino acids have a central carbon (C), at least one amino group ($-\text{NH}_2$), at least one carboxy (acid) group ($-\text{COOH}$), and a side chain (R group) that makes each amino acid unique. The generic amino acid may be represented as follows:



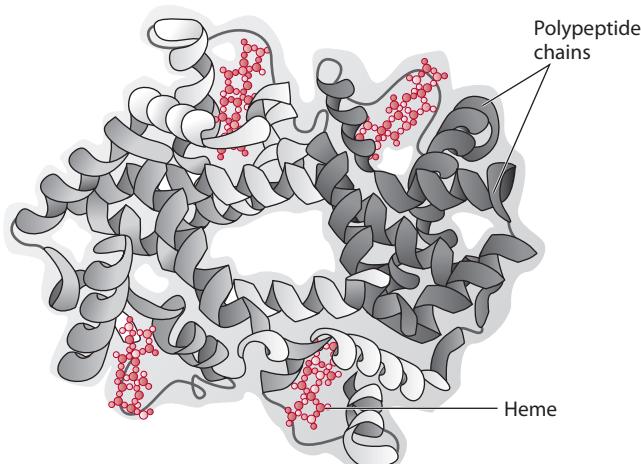
Depending on the pH of the environment, the amino and carboxy groups can accept or donate H^+ , as shown in Figure 6.5. The distinctive characteristics of the side chains of the amino acids that make up a polypeptide bestow on a protein its structure, and consequently its functional role in the body. These same distinctive characteristics determine whether certain amino acids can be synthesized in the body or must be ingested. Furthermore, these characteristics program the various amino acids for their specific metabolic pathways in the body. The differences among the side chains of the amino acids commonly found in body proteins are shown in the structural classification of amino acids in Table 6.1 on page 186. This division of amino acids based on structural similarities is one approach used to classify amino acids. Dividing



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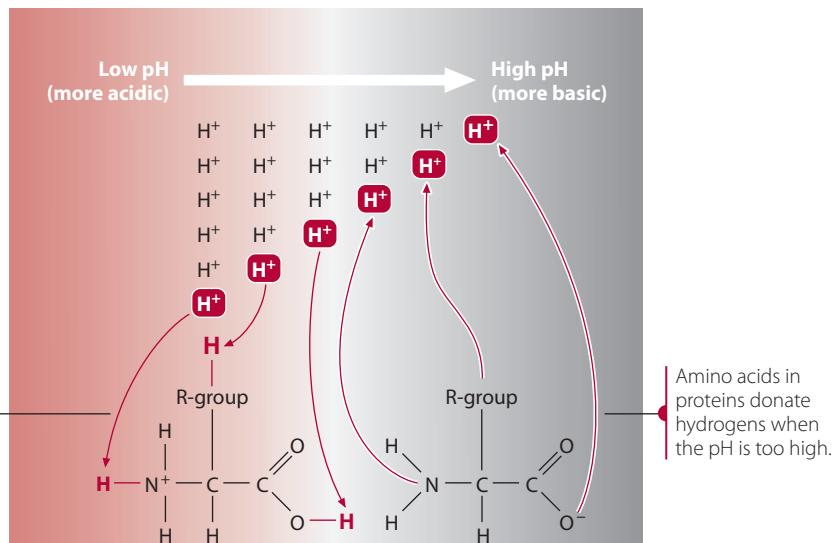
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**Figure 6.4** Quaternary structure of the hemoglobin protein.

amino acids based on the presence or absence of net charge is another way to classify amino acids.

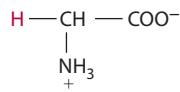
NET ELECTRICAL CHARGE

The amino acids listed in Tables 6.1, 6.2a, and 6.2b and discussed here are shown based on their structures as they exist in an aqueous solution at the physiological pH, approximately 6 to 8, of the human body. Amino acids in aqueous solution are ionized. The term **zwitterion**, or dipolar ion, is applied to amino acids with no carboxy or amino groups in their side chain to generate an additional charge to the molecule. Zwitterions have no net electrical charge, because their side chains are not charged, and the one positive and one negative charge from the amino and carboxy group, respectively, in their

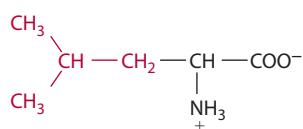
**Figure 6.5** The role of the amino acid in pH balance.**Table 6.1** Structural Classification of Amino Acids

1. With aliphatic side chains

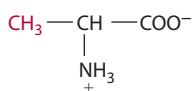
Glycine (Gly)



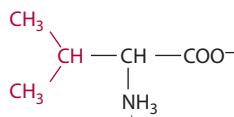
Leucine (Leu)



Alanine (Ala)

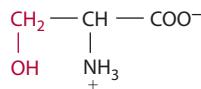


Valine (Val)

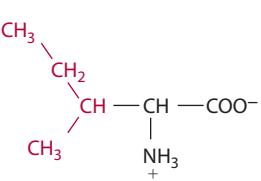


2. With side chains containing hydroxylic (OH) groups*

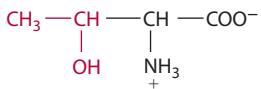
Serine (Ser)



Isoleucine (Ile)



Threonine (Thr)

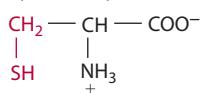


*Although tyrosine contains a hydroxyl group, it is classified as an amino acid containing an aromatic ring (see Group 6).

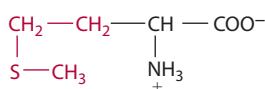
Table 6.1 (Continued)

3. With side chains containing sulfur atoms

Cysteine (Cys)

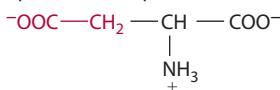


Methionine (Met)

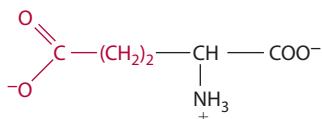


4. With side chains containing acidic groups or their amides

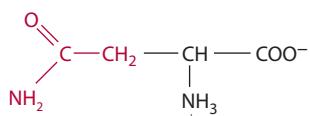
Aspartic acid (Asp)



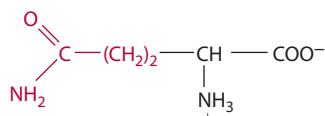
Glutamic acid (Glu)



Asparagine (Asn)

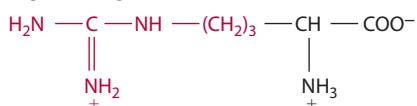


Glutamine (Gln)

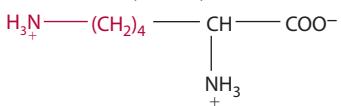


5. With side chains containing basic groups

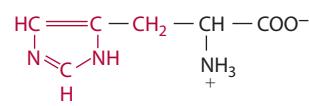
Arginine (Arg)



Lysine (Lys)

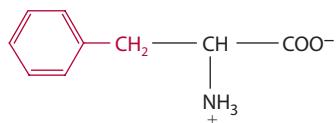


Histidine (His)

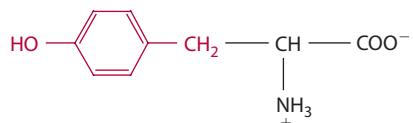


6. With side chains containing aromatic ring

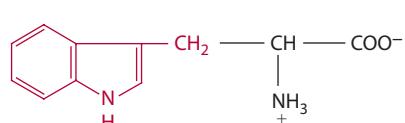
Phenylalanine (Phe)



Tyrosine (Tyr)

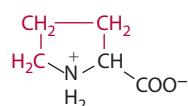


Tryptophan (Trp)



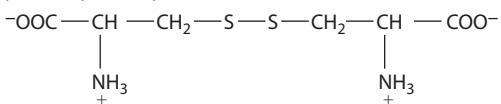
7. Imino acids

Proline (Pro)

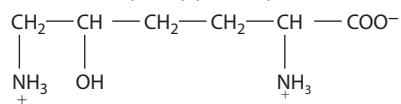


8. Amino acids formed posttranslationally

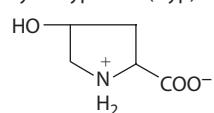
Cystine (Cys-S-S-Cys)



Hydroxylysine (Hyl)



Hydroxyproline (Hyp)



3-methylhistidine (3-meHis)

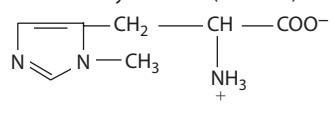


Table 6.2a

Neutral Amino Acids

Alanine	Glycine	Phenylalanine	Tryptophan
Asparagine	Isoleucine	Proline	Tyrosine
Cysteine	Leucine	Serine	Valine
Glutamine	Methionine	Threonine	

Table 6.2b

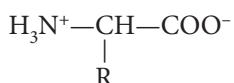
Negatively Charged Amino Acids

Aspartic acid
Glutamic acid

Positively Charged Amino Acids

Arginine
Histidine
Lysine

base structure cancel each other out. Amino acids with no net charge do not migrate substantially if placed in an electric field.



Amino acids with neutral side chains have no net electrical charge, are shown in Table 6.2a.

Two groups of amino acids (Table 6.2b) exhibit a net charge. Because of the presence of additional carboxyl groups in the side chains, the dicarboxylic amino acids aspartic acid and glutamic acid exhibit a net negative charge at pH 7; these forms of the amino acids are called aspartate and glutamate. Amino acids or proteins with a high content of dicarboxylic amino acids exhibit a negative charge and migrate toward the anode if placed in an electric field. In contrast, because of the presence of additional amino groups in their side chains, the basic amino acids (lysine, arginine, histidine), also called dibasic, exhibit a net positive charge at pH 7.

POLARITY

The tendency of an amino acid to interact with water at physiological pH—that is, its polarity—represents another means of classifying amino acids. Polarity depends on the side chain or R group of the amino acid. Amino acids are classified as polar or nonpolar, although they can have varying levels of polarity. Both the dicarboxylic (aspartic acid and glutamic acid) and basic (lysine, arginine, histidine) amino acids are polar; that is, they interact with water. The neutral amino acids interact with water to different degrees and can be divided into polar (both neutral and charged), nonpolar, and relatively nonpolar categories, as listed in Table 6.2c.

The polar neutral amino acids contain functional groups in their side chains, such as the hydroxyl group for serine and threonine, the sulfur atom for cysteine, and the amide group for asparagine and glutamine, that can interact through hydrogen bonds with water (the aqueous environment of cells); thus, we categorize them as polar. Polar charged amino acids also interact with

aqueous environments and can form salt bridges or can interact with electrolytes and minerals such as potassium, chloride, and phosphate. Hence, polar amino acids are found on the surfaces of proteins, or, if oriented inward, the polar amino acids often function at a protein's (such as an enzyme's) binding site. In contrast, the nonpolar amino acids listed in the third column of Table 6.2c contain side chains that do not interact with water. Thus, these amino acids typically do not interact with water and are categorized as nonpolar or hydrophobic (water fearing). The aromatic amino acids are considered relatively nonpolar. Tyrosine, for example, because of its hydroxyl group on the phenyl ring, can to a limited extent form hydrogen bonds with water—hence the term “relatively nonpolar.” Because they do not interact with water, the nonpolar (and often the relatively nonpolar) amino acids are typically found compacted (attracted by van der Waal forces, for example) and oriented toward or within the central region or core portion of proteins.

ESSENTIALITY

While amino acids can be classified based on structure or properties such as net charge or polarity, in 1957 Rose [1] categorized the amino acids found in proteins as nutritionally essential (indispensable) or nutritionally nonessential (dispensable). At that time, only eight amino acids—leucine, isoleucine, valine, lysine, tryptophan, threonine, methionine, and phenylalanine—were considered essential for adult humans. Histidine was later added as an essential amino acid. We now know that if we give an α -keto or hydroxy acid of leucine, isoleucine, valine, tryptophan, methionine, or phenylalanine (as may be done with people with renal failure), the α -keto or hydroxy acid form of these amino acids can be transaminated to form the respective amino acid. Three amino acids—lysine, threonine, and histidine—cannot undergo transamination to any appreciable extent. Thus, lysine, threonine, and histidine are totally indispensable. Table 6.2d shows the essential amino acids.

Identifying amino acids strictly as dispensable or indispensable is an inflexible classification, however, that allows no gradations, even in decidedly different or changing physiological circumstances. Newer categories added to the essential/indispensable and nonessential/dispensable categories include conditionally or acquired indispensable amino acids. A dispensable amino acid may become indispensable if an organ fails to function properly, as in the case of infants born prematurely or in the case

Table 6.2c

Polar Neutral Amino Acids	Polar Charged Amino Acids	Nonpolar Neutral Amino Acids	Relatively Nonpolar Amino Acids
Asparagine	Arginine	Alanine	Phenylalanine
Cysteine	Lysine	Glycine	Tryptophan
Glutamine	Histidine	Isoleucine	Tyrosine
Serine	Glutamate	Leucine	
Threonine	Aspartate	Methionine	
		Proline	
		Valine	

Table 6.2d

Essential / Indispensable Amino Acids		
Phenylalanine	Methionine	Isoleucine
Valine	Tryptophan	Leucine
Threonine	Histidine	Lysine

Table 6.2e Conditionally Indispensable Amino Acids and Their Precursors

Amino Acid	Precursor(s)
Tyrosine	Phenylalanine
Cysteine	Methionine, serine
Proline	Glutamate
Arginine	Glutamine or glutamate, aspartate
Glutamine	Glutamate, ammonia

of disease-associated organ malfunction. For example, neonates born prematurely often have immature organ function and are unable to synthesize many nonessential amino acids, such as cysteine and proline. Immature liver function or liver malfunction caused by cirrhosis, for example, impairs phenylalanine and methionine metabolism, which occurs primarily in the liver. Consequently, the amino acids tyrosine and cysteine, normally synthesized from phenylalanine and methionine catabolism, respectively, become indispensable until normal organ function is established. Inborn errors of amino acid metabolism, which result from genetic disorders in which key enzymes in amino acid metabolism lack sufficient enzymatic activity, illustrate another situation in which dispensable amino acids become indispensable. People with classical phenylketonuria (PKU) exhibit little to no phenylalanine hydroxylase activity. This enzyme converts phenylalanine to tyrosine. Without hydroxylase activity, tyrosine is not synthesized in the body and must be provided completely by diet; thus, it is indispensable for people with this condition (PKU). In other inborn errors of metabolism, amino acids such as cysteine become indispensable. Thus, amino acids that normally are dispensable may become indispensable under certain physiological conditions. Amino acids that are conditionally indispensable are listed in Table 6.2e, along with their usual amino acid precursors.

Sources of Protein

Exogenous proteins, following ingestion, serve as sources of the essential amino acids and are the primary source of the additional nitrogen needed to synthesize the nonessential amino acids and nitrogen-containing compounds. Dietary or exogenous sources of protein include:

- animal products such as meat, poultry, fish, and dairy products (with the exception of butter, sour cream, and cream cheese)
- plant products such as grains, grain products, legumes, and vegetables

Protein quality is discussed at the end of this chapter.

Endogenous proteins presented to the digestive tract represent another source of amino acids and nitrogen, and

they mix with exogenous nitrogen sources. Endogenous proteins include:

- desquamated mucoasal cells, which generate about 50 g of protein per day
- digestive enzymes and glycoproteins, which generate about 17 g of protein each day [2]

The digestive enzymes and glycoproteins are derived from digestive secretions of the salivary glands, stomach, intestine, biliary tract, and pancreas. Most of these endogenous proteins, which may total about 70 g or more per day, are digested and provide amino acids available for absorption. Digestion of protein and absorption of amino acids are crucial for optimal protein nutriture.

Digestion and Absorption

This section of the chapter focuses first on digestion of protein in the digestive tract. Next, it reviews absorption: the mechanisms by which the end products of protein digestion are transported across the brush border (also called apical) membrane of the intestinal cell. Amino acids must cross the basolateral (also called serosal) membrane and leave the intestinal cell to gain access to the blood, so they can be circulated to tissues. Therefore, the transport systems that ferry the amino acids across the basolateral membrane and other extraintestinal tissue membranes are presented in this section. Lastly, because not all amino acids entering the intestinal cells get into the blood, the section covers the use of amino acids by the intestinal cells themselves.

PROTEIN DIGESTION

Macronutrient digestion in general terms was covered in Chapter 2. This chapter outlines digestion with respect solely to protein within the gastrointestinal tract organs (Figure 6.6 and Table 6.3) and covers some of the major enzymes responsible for protein digestion.

Mouth and Esophagus

No appreciable digestion of protein occurs in the mouth or esophagus.

Stomach

The digestion of exogenous protein begins in the stomach, with the action of hydrochloric acid (HCl) in gastric juice (which has a pH of about 1–2). Hydrochloric acid release from gastric parietal (also called oxytic) cells is stimulated by a variety of compounds, including the hormone gastrin, the neuropeptide gastrin-releasing peptide (GRP), the neurotransmitter acetylcholine, and the amine histamine. Hydrochloric acid denatures (disrupts) the quaternary,

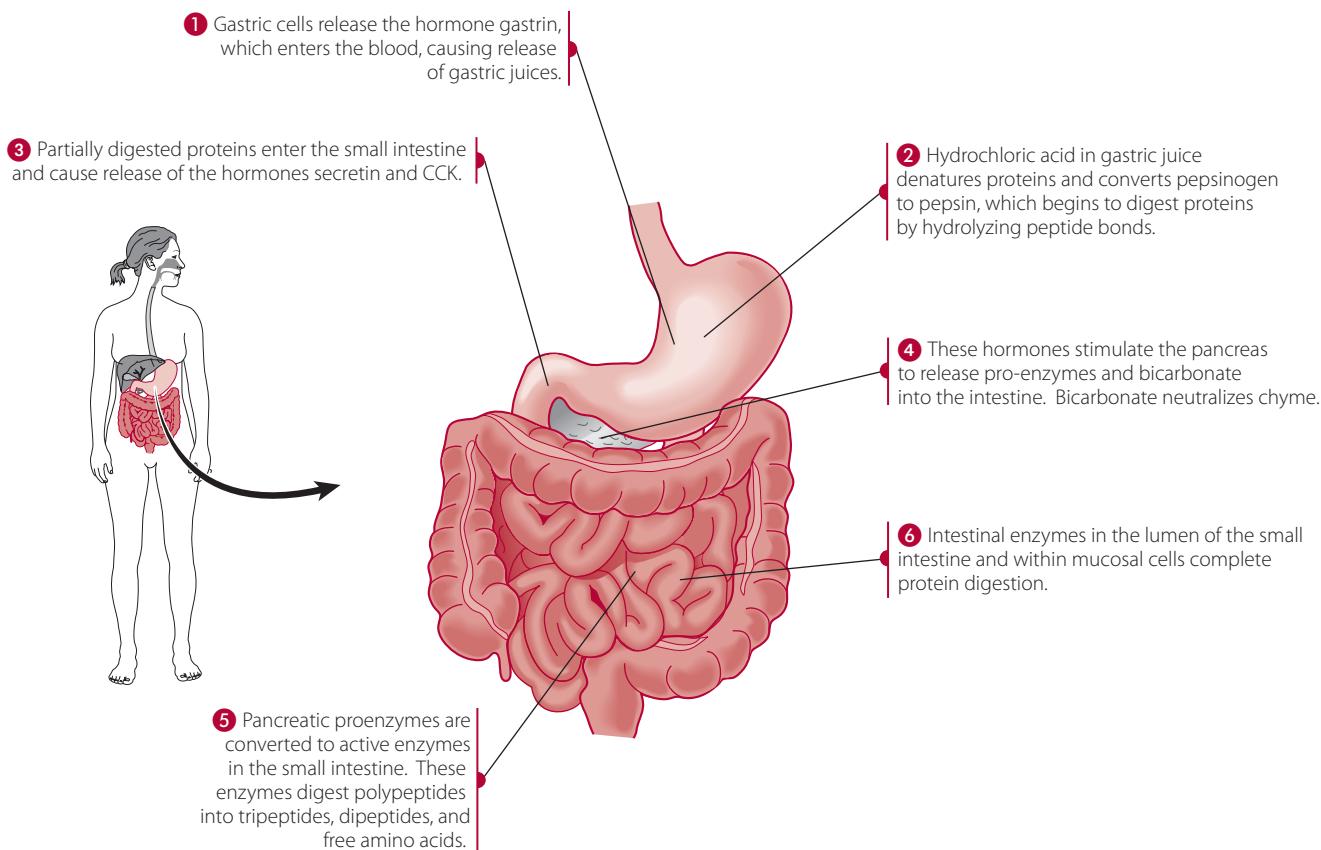


Figure 6.6 An overview of protein digestion.

tertiary, and secondary structures of protein and begins the activation of pepsinogen to pepsin, which is secreted by gastric chief cells. Denaturants such as hydrochloric acid break apart hydrogen and electrostatic bonds to unfold or uncoil the protein; however, peptide bonds are not affected by the hydrochloric acid. Pepsin, once formed, is catalytic against pepsinogen as well as other proteins.



Pepsin functions as an endopeptidase at a pH <~3.5 to hydrolyze peptide bonds in proteins or polypeptides. Pepsin attacks peptide bonds adjacent to the carboxyl end of a relatively wide variety of amino acids (i.e., pepsin has low specificity), including leucine; methionine; aromatic amino acids consisting of phenylalanine, tyrosine, and tryptophan; and the dicarboxylic amino acids glutamate and aspartate. The end products of gastric protein digestion with pepsin include primarily large polypeptides, along with some oligopeptides and free amino acids. These

Table 6.3 Some Enzymes Responsible for the Digestion of Protein

Zymogen	Enzyme or Activator	Enzyme	Site of Activity	Substrate (peptide bonds adjacent to)
Pepsinogen	HCl or pepsin	Pepsin	Stomach	Most amino acids, including aromatic, dicarboxylic, leu, met
Trypsinogen	Enteropeptidase or Trypsin	Trypsin	Intestine	Basic amino acids
Chymotrypsinogen	Trypsin	Chymotrypsin	Intestine	Aromatic amino acids, met, asn, his
Procarboxypeptidases	Trypsin	Carboxypeptidase A B Aminopeptidases	Intestine	C-terminal neutral amino acids C-terminal basic amino acids N-terminal amino acids

end products are emptied in an acid chyme through the pyloric sphincter into the duodenum (the proximal or upper part of the small intestine) for further digestion.

Small Intestine

The end products in the acid chyme that are delivered into the duodenum further stimulate the release of regulatory hormones and peptides such as secretin and cholecystokinin (CCK) from the mucosal endocrine cells. Secretin and CCK are carried by the blood to the pancreas, where the acinar cells are stimulated to secrete alkaline pancreatic juice containing bicarbonate, electrolytes, water, and digestive proenzymes, also called zymogens. In addition to pancreatic juice, the Brunner's glands of the small intestine release mucus-rich secretions.

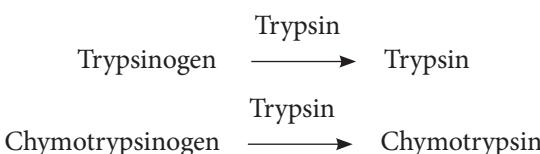
Digestive proenzymes or zymogens secreted by the pancreas, and further responsible for protein and polypeptide digestion, include:

- trypsinogen
- chymotrypsinogen
- procarboxypeptidases A and B
- proelastase
- collagenase

Within the small intestine, these inactive zymogens must be chemically altered to be converted into their respective active enzymes, capable of substrate hydrolysis. The following reactions occur in the small intestine to activate the zymogens:



Enteropeptidase (an endopeptidase formerly known as enterokinase) is secreted from the intestinal brush border in response to CCK and secretin. Once trypsin is formed, it can act on more trypsinogen as well as on chymotrypsinogen to yield active proteolytic enzymes.



Trypsin and chymotrypsin are both endopeptidases. Trypsin is specific for peptide bonds adjacent to basic amino acids (lysine and arginine). Excess free trypsin generated from trypsinogen also acts by negative feedback to inhibit trypsinogen synthesis by pancreatic cells, thereby regulating pancreatic zymogen secretion [2]. Chymotrypsin is specific for peptide bonds adjacent to aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and for peptide bonds adjacent to methionine, asparagine, and histidine.

Both elastase, an endopeptidase derived from proelastase, and collagenase hydrolyze polypeptides into smaller fragments, such as oligopeptides and tripeptides. Procarboxypeptidases are converted to carboxypeptidases by trypsin and serve as exopeptidases.



These exopeptidases attack peptide bonds at the carboxy (C)-terminal end of polypeptides to release free amino acids. Carboxypeptidases are zinc-dependent enzymes and specifically require zinc at the active site. Carboxypeptidase A hydrolyzes peptides with C-terminal aromatic neutral or aliphatic neutral amino acids. Carboxypeptidase B cleaves basic amino acids from the C-terminal end, generating free basic amino acids as end products.

Several peptidases are produced by the brush border of the small intestine, including the ileum, enabling peptide digestion and amino acid absorption to occur in the distal small intestine. Some of these peptidases include:

- aminopeptidases, which vary in specificity and cleave amino acids from the amino (N)-terminal end of oligopeptides
- dipeptidylaminopeptidases, some of which are magnesium-dependent and hydrolyze N-terminal amino acids from dipeptides
- tripeptidases, which are specific for selected amino acids and hydrolyze tripeptides to yield a dipeptide and a free amino acid

Some tripeptides, such as trileucine, undergo hydrolysis at the brush border, whereas other tripeptides, such as triglycine or proline-containing peptides, are absorbed intact and hydrolyzed within the intestinal cell. Amino acids (an end product of protein digestion) have also been shown to inhibit the activity of brush border peptidases (a process called end product inhibition).

Protein digestion yields two main end products: peptides, principally dipeptides and tripeptides, and free amino acids. To be used by the body, these end products must now be absorbed across the brush border of the intestinal epithelial mucosal cells (also called enterocytes).

INTESTINAL BRUSH BORDER MEMBRANE AMINO ACID AND PEPTIDE ABSORPTION

Absorption represents the passage of a substance (such as an amino acid or peptide) from the lumen of the gastrointestinal tract, most often the intestine, across the brush border (also called apical) membrane of the intestinal cell and into the cell. This section addresses the mechanisms by which amino acids and peptides are absorbed into intestinal cells.

Amino Acid Transport

Amino acid absorption occurs along the entire small intestine; however, most amino acids are absorbed in the proximal (upper) small intestine. Absorption of amino acids into the intestinal cells requires carriers; however, paracellular absorption also can occur. Transport systems for amino acids have been traditionally designated using a lettering system with a further distinction that uppercase letters be used for sodium dependence and lowercase letters for sodium independence [3]; however, not all systems (e.g., the L, which is sodium-independent) follow this rule. Moreover, with most of the systems now cloned, amino acid transporters are being reclassified and characterized in further detail. Table 6.4 lists some of the transport systems responsible for carrying amino acids across the brush border into the intestinal cell and some examples of amino acids that are carried by each of these transport systems. The transporters vary in mechanism of action. Some transporters, such as the y^+ , t, asc, $b^{0,+}$, and x_c^- , are passive and function as exchangers or uniporters. Other carriers are active and are driven by one or more transmembrane ion gradients. For example, with the X_{AG}^- transporter (antiporter), glutamate, H^+ and 3 Na^+ enter the cell in exchange for 1 K^+ , and with the N system, glutamine and Na^+ enter the cell in exchange for H^+ . Most amino acids are thought to be transported across the intestinal cell brush border membrane by sodium-dependent transporters as shown in Figure 6.7 [4]. This transport involves first sodium binding to the carrier. This binding of sodium appears to increase the affinity of the carrier for the amino acid, which then binds to the carrier.

Table 6.4 Some Systems Transporting Amino Acids across the Intestinal Cell Brush Border Membrane

Amino Acid Transport Systems	Sodium Required	Examples of Substrates Carried
L	No	Leucine, other neutral amino acids
B	Yes	Phenylalanine, tyrosine, tryptophan, isoleucine, leucine, valine
IMINO	Yes	Proline, glycine
y^+	No	Basic amino acids
X_{AG}^-	Yes	Aspartate, glutamate
$b^{0,+}$	Yes	Most neutral and basic amino acids
$b^{0,+}$	No	Most neutral and basic amino acids
y^{+L}	No/Yes	Basic and neutral amino acids
ASC	Yes	Alanine, serine, cysteine
t	No	Tryptophan, phenylalanine, tyrosine
asc	No	Similar to ASC
N	Yes	Glutamine, asparagine, histidine
ag	No	Aspartate, glutamate

However, for the transport of some amino acids, these first two steps may be reversed; that is, the binding of the amino acid may precede the binding of the sodium. Once the sodium–amino acid–cotransporter complex forms, a conformational change in the complex results in the delivery of the sodium and amino acid into the cytoplasm of the enterocyte. Lastly, sodium is pumped out of the cell by Na^+/K^+ -ATPase.

Competition between amino acids for transport by a common carrier has been documented. In addition, regulation (both induced de novo synthesis of specific amino acid carriers and decreased carrier synthesis) of transport carriers has been shown and helps to ensure adequate capacity [5,6].

The affinity (K_m) of a carrier for an amino acid is influenced both by the hydrocarbon mass of the amino acid's side chain and by the net electrical charge of the amino acid. As the hydrocarbon mass of the side chain increases, affinity increases [7]. Thus, the branched-chain amino acids typically are absorbed faster than smaller amino acids. Neutral amino acids also tend to be absorbed at higher rates than dibasic (basic) or dicarboxylic (acidic) amino acids. Essential (indispensable) amino acids are absorbed faster than nonessential (dispensable) amino acids, with methionine, leucine, isoleucine, and valine being the most rapidly absorbed [7]. The most slowly absorbed amino acids are the two dicarboxylic (acidic) amino acids, glutamate and aspartate, both of which are nonessential [7].

Ingesting free, crystalline L-amino acids is thought by many athletes to be superior to ingesting natural foods containing protein for muscle protein synthesis. However, amino acids using the same carrier system compete with each other for absorption. Thus, ingesting one amino acid or a particular group of amino acids that use the same carrier system may create, depending on the amount ingested, a competition between the amino acids for absorption. The result may be that the amino acid present in highest concentration is absorbed but also may impair the absorption of the other, less concentrated amino acids carried by that same system. Thus, amino acid supplements may result in impaired or imbalanced amino acid absorption. Furthermore, absorption of peptides (which are obtained from digestion of natural protein-containing foods) is more rapid than absorption of an equivalent mixture of free amino acids. Also, nitrogen assimilation following ingestion of protein-containing foods is superior to that following ingestion of free amino acids. In other words, free amino acids have no absorptive advantage. Moreover, the supplements are usually expensive, typically taste terrible, and may cause gastrointestinal distress.

Peptide Transport

Peptide (primarily dipeptide and tripeptide) transport across the brush border membrane of the enterocyte occurs by a transport system different from those that transport amino acids. One transport system designated

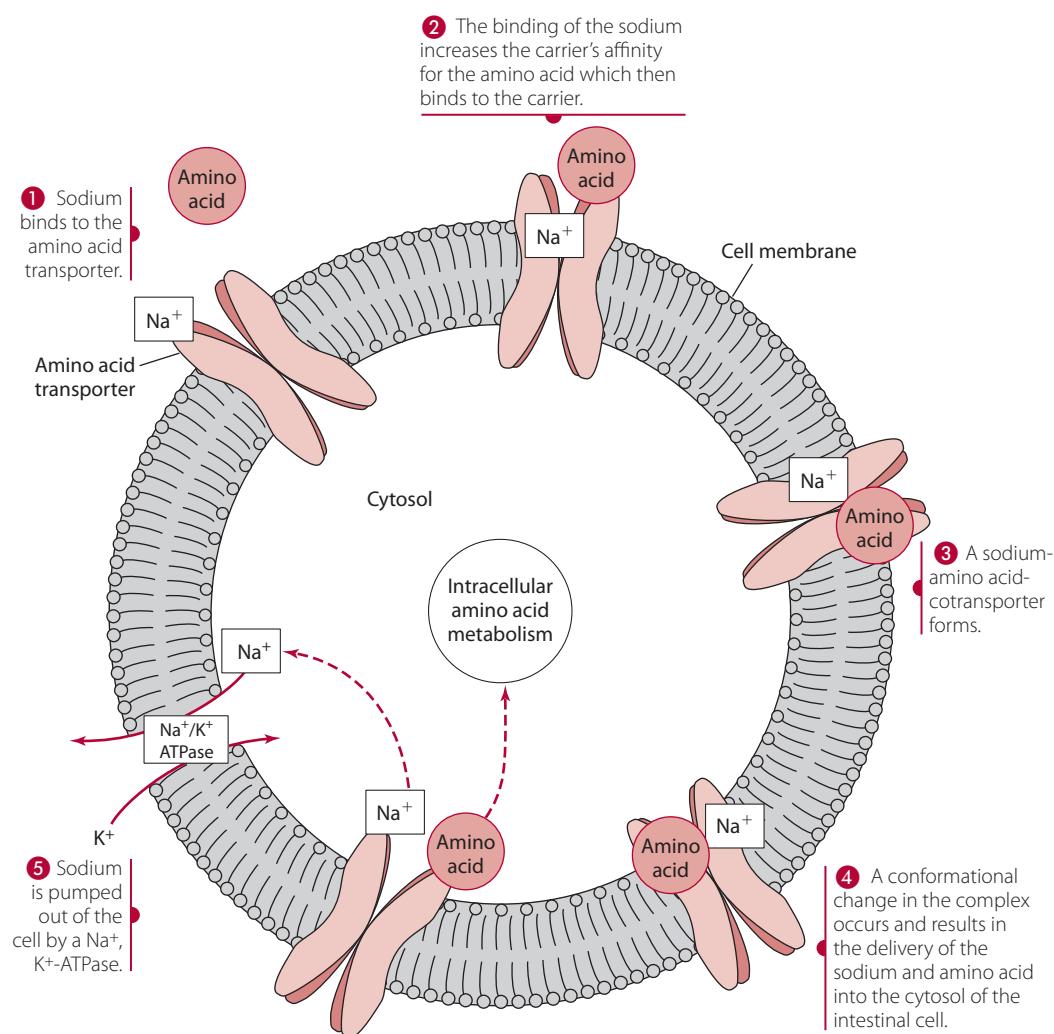
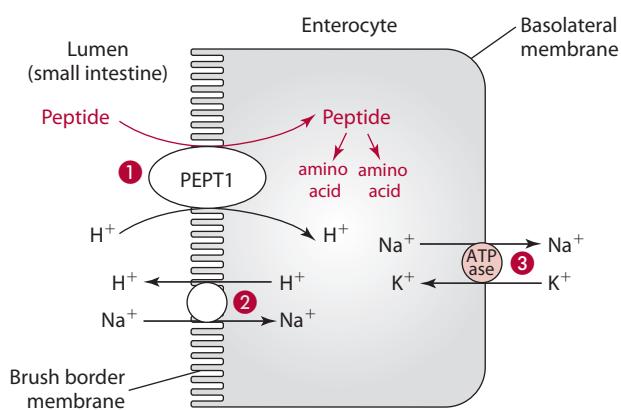


Figure 6.7 Sodium (Na^+) dependent transport of an amino acid into a cell.

PEPT1 appears to transport all di- and tripeptides across the brush border of intestinal cells [6,8,9]. The transport of peptides across the brush border membrane using PEPT1 is associated with the comovement of protons (H^+) and thus depolarization of the brush border membrane. An area of low pH lying adjacent to the brush border surface of the enterocyte provides the driving force for the H^+ gradient. Thus, as shown in Figure 6.8, as the dipeptide or tripeptide is transported into the enterocyte, an H^+ ion also enters the enterocyte. The transport of the H^+ into the enterocyte results in an intracellular acidification. The H^+ ions are pumped back out into the lumen in exchange for Na^+ ions. An Na^+/K^+ -ATPase allows for Na^+ extrusion at the basolateral membrane to maintain the gradient [8, 9].

Affinity of the carrier for the peptide appears to be influenced by stereoisomerism, the length of the side chain of the N-terminal amino acid, substitutions on the N- and C-terminals, and the number of amino acids in the peptide [10]. For example, as the length of the peptide increases above three amino acids, affinity for transport decreases. In addition, peptides, like amino acids, compete with one another for transporters.



- 1 Peptides are transported into the intestinal cell along with H^+ .
- 2 The H^+ are pumped back into the intestinal lumen in exchange for Na^+ .
- 3 A Na^+/K^+ -ATPase pump pumps Na^+ out of the cell in exchange for K^+ across the basolateral membrane.

Figure 6.8 Peptide transport. Peptides are transported across the brush border membrane of the intestinal epithelial mucosal cell.

Peptide transport is thought to occur more rapidly than amino acid transport and is thought to represent the primary system for amino acid absorption. Over 60% of amino acids are absorbed in the form of small peptides, with the remaining absorbed as free amino acids [11,12]. Peptides, once within the enterocytes, are generally hydrolyzed by cytoplasmic peptidases to generate free intracellular amino acids; however, small peptides have been found intact in circulation.

INTESTINAL BASOLATERAL MEMBRANE TRANSPORT OF AMINO ACIDS

The transport of the amino acids across the basolateral membrane of the enterocyte into the interstitial fluid appears to be the same as the transport of amino acids across the membrane of nonepithelial cells. Diffusion and sodium-independent transport are thought to be the primary modes of amino acid transport across the basolateral membrane of the enterocyte. Sodium-dependent pathways are quantitatively important when the amino acid concentrations in the gut lumen are low. Active transport of amino acids into the enterocytes is necessary to provide the enterocyte with amino acids to meet its own needs. Some of the basolateral transport systems include those listed in Table 6.5.

The significance of the amino acid transporters becomes extremely apparent when people are born without the ability to make a properly working transporter due to a genetic defect. Lysinuric protein intolerance results from defects in the cationic transporters in the intestine, liver, and kidney. The defects cause poor absorption of lysine, arginine, and ornithine into the body and thus low plasma concentrations and availability of these amino acids for protein synthesis and for urea cycle activity. Symptoms of the disorder include hyperammonemia, growth retardation, muscle weakness, hepatomegaly, and hypotonia,

among other problems. Nutrition support involves a protein-restricted diet to deal with the hyperammonemia and supplements of citrulline to help improve arginine and ornithine production in the body. Lysine and arginine supplements have not been effective. A second example of the critical role of a transporter is illustrated by the condition Hartnup disease, an autosomal recessive genetic disorder, that affects absorption of tryptophan and other neutral amino acids (likely the B transport system) into intestinal and kidney cells. People with Hartnup disease malabsorb tryptophan and other amino acids and often develop a niacin deficiency without treatment with large doses of niacin (remember tryptophan is a precursor of niacin).

INTESTINAL CELL AMINO ACID USE

Although the preceding sections have covered amino acid absorption across the brush border and basolateral membranes, it must be remembered that not all amino acids are transported out of the intestinal cell and into circulation. Many of the amino acids absorbed following protein digestion are used by the intestinal cells for energy or synthesis of proteins and other nitrogen-containing compounds including:

- structural proteins for new intestinal cells
- nucleotides
- apoproteins necessary for lipoprotein formation
- new digestive enzymes
- hormones
- nitrogen-containing compounds

In addition, amino acids may be partially metabolized either to other amino acids or to other compounds that may be released into portal blood. It is estimated that the intestine uses 30–40% and splanchnic tissues use up to 50% of some of the essential amino acids absorbed from the diet [13]. Moreover, the intestines are thought to use up to about 90% of glutamate absorbed from the diet [13]. The next several paragraphs discuss the metabolism of glutamine, glutamate, aspartate, arginine, methionine, and cysteine in intestinal cells.

Intestinal Glutamine Metabolism

Glutamine is used in several ways by the intestinal cell (Figure 6.9). It is degraded extensively by intestinal cells as a primary source of energy. It also has been shown to have trophic (growth) effects, such as stimulating cell proliferation, on the gastrointestinal mucosa cells [13,14]. Glutamine helps to prevent atrophy of gut mucosa and bacterial translocation. In addition, glutamine has been shown to enhance the synthesis of heat shock proteins. It is also needed in large quantities along with threonine for the synthesis of mucins found in gastrointestinal tract mucus secretions. These roles of glutamine in the gastrointestinal tract have

Table 6.5 Some Systems Transporting Amino Acids across the Intestinal Cell Basolateral Membrane

Amino Acid Transport System	Sodium Required	Examples of Substrates Carried
L	No	Leucine, other neutral amino acids
y ⁺	No	Basic amino acids
b ^{0,+}	No	Neutral and basic amino acids
t	No	Phenylalanine, tyrosine, tryptophan
X _{AG} ⁻	Yes	Aspartate and glutamate
A	Yes	Alanine, other short-chain, polar, neutral amino acids
ASC	Yes	Alanine, cysteine, serine, other three- and four-carbon amino acids
asc	No	Same substrates as ASC
GLY	Yes	Glycine

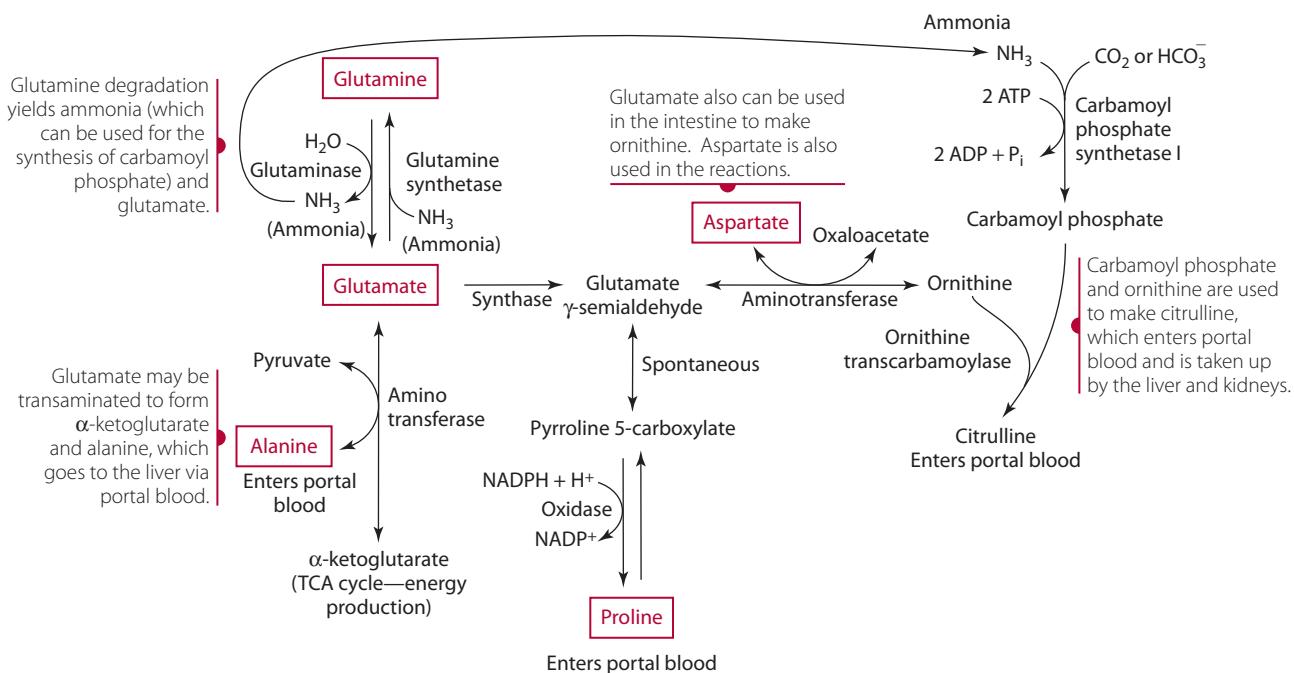
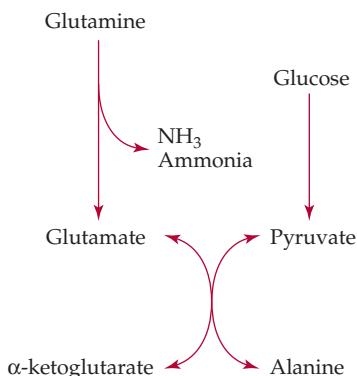


Figure 6.9 A partial overview of amino acid metabolism in the intestinal cell.

prompted several companies to enrich enteral and parenteral (intravenous) nutrition products with glutamine. When glutamine is fed through tube feedings, over 50% of glutamine is extracted by the splanchnic (visceral) bed. It is estimated that the human gastrointestinal tract uses up to 10 g glutamine per day, and that the immune system uses over 10 g per day. In addition to dietary glutamine, much of the body's glutamine that is produced by the skeletal muscles (and to lesser extents by the lungs, brain, heart, and adipose) is released and taken up mostly by the intestinal cells.

Glutamine not used for energy production within the intestine also may be partially catabolized to generate ammonia and glutamate. The ammonia enters the portal blood for uptake by the liver or may be used within the intestinal cell for carbamoyl phosphate synthesis. Glutamate may undergo transamination (in which its amino group is removed) to form α -ketoglutarate, an intermediate in the tricarboxylic acid (TCA), also referred to as the Krebs, cycle. The amino group is transferred to the compound pyruvate (which is present in the intestinal cell from glucose metabolism) to form the amino acid alanine, as shown below.



Once formed, alanine leaves the intestinal cell, enters portal blood, and subsequently is taken up along with ammonia by periportal hepatocytes (liver cells). The periportal hepatocytes funnel the ammonia into urea synthesis.

Intestinal Glutamate Metabolism

Glutamate is obtained directly from diet or generated from glutamine deamination within the intestinal cell. Glutamate is often transaminated with pyruvate to form α -ketoglutarate and alanine (Figure 6.9). Glutamate (in the intestinal cell) not used for alanine synthesis can be used in the intestinal cell to synthesize proline, as shown here:



Proline is then released into portal blood for delivery to the liver. Glutamate not used for proline synthesis may be used along with aspartate to synthesize ornithine, which in turn may be released into portal blood or can be used to make citrulline (Figure 6.9).

Glutamate is also used with glycine and cysteine to make the tripeptide glutathione in the enterocyte (and other body cells). Glutathione (Figure 6.10) functions as an antioxidant; it reduces many reactive oxygen species (e.g., O₂[•] and OH[•]) and lipid (LOOH) and hydrogen (H₂O₂) peroxides in the intestinal cell. Unless these reactive species are destroyed, they can damage cellular DNA, proteins, and polyunsaturated fatty acids in intestinal cell membranes to cause membrane peroxidation and cell necrosis (death). Glutathione is discussed in more detail later in this

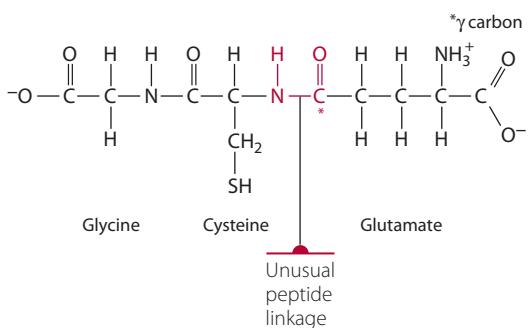
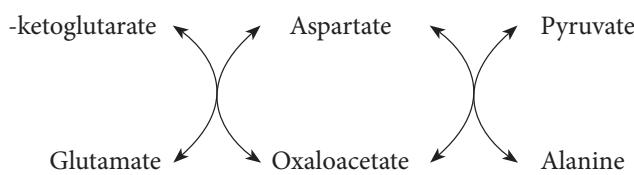


Figure 6.10 The structure of reduced glutathione (GSH).

chapter under “Synthesis of Plasma Proteins, Nitrogen-Containing Nonprotein Compounds, and Purine and Pyrimidine Bases.”

Intestinal Aspartate Metabolism

In addition to metabolism of glutamine and glutamate, metabolism of aspartate from the diet generally occurs within intestinal cells. Aspartate most often undergoes transamination with α -ketoglutarate or pyruvate to generate oxaloacetate and either glutamate or alanine, respectively.



Very little aspartate is found in portal blood.

Intestinal Arginine Metabolism

Arginine is also taken up by intestinal cells. Up to 40% of dietary arginine is oxidized in intestinal cells yielding citrulline and urea [15]. Citrulline is also made in intestinal cells from carbamoyl phosphate and ornithine. Two enzymes (carbamoyl phosphate synthetase I and ornithine transcarbamoylase) and possibly all five enzymes of the urea cycle are present in intestinal cells and are responsible for carbamoyl phosphate and ornithine synthesis [16]. Carbamoyl phosphate synthetase I catalyzes the synthesis of carbamoyl phosphate from ammonia (NH_3), carbon dioxide (CO_2) or bicarbonate (HCO_3^-), and ATP in mucosal epithelial cells, as shown in Figure 6.9 and here:



Ornithine transcarbamoylase synthesizes citrulline from ornithine and carbamoyl phosphate as follows:



Citrulline, once made, is released into blood and then typically taken up by the kidney, which uses it for arginine synthesis. In fact, the kidney is the main organ responsible

for provision of arginine to body tissues. Not all citrulline, however, goes to the kidney; the liver takes up some citrulline released from intestinal cells.

Intestinal Methionine and Cysteine Metabolism

Methionine and cysteine also appear to be metabolized by intestinal cells. Studies suggest that up to 52% of methionine intake is metabolized in the gut [17]. Cysteine, generated from methionine or obtained directly from diet, is used to make glutathione. Alternately, cysteine can be metabolized primarily (70%–90%) through cysteine sulfinate to taurine, and to a lesser extent (10%–30%) from cysteine sulfinate to pyruvate and sulfite [17]. The methionine and cysteine degradative pathways are shown later in Figure 6.30 and described in more detail under the section “Hepatic Catabolism and Uses of Sulfur (S)-Containing Amino Acids.”

AMINO ACID ABSORPTION INTO EXTRATESTINAL TISSUES

Amino acids not used by the intestinal cell are transported across the basolateral membrane of the enterocyte into interstitial fluid, where they enter the capillaries of the villi and eventually the portal vein for transport to the liver. Most peptides that have been absorbed intact into the intestinal cell undergo hydrolysis by proteases present within the cytoplasm of the enterocyte. Thus, primarily free amino acids are found in portal circulation. Occasionally, however, small peptides can be found in splanchnic circulation and are thought to have entered circulation by paracellular or intercellular routes—that is, by passing through tight junctions of mucosal cells or by transcellular endocytosis [18]. With illnesses, especially affecting the intestines (such as inflammatory bowel diseases or celiac disease), the gastrointestinal tract can become more permeable thus increasing the likelihood of peptides appearing intact in the blood.

The ability to administer peptides that can be used by body tissues directly into the blood (parenteral nutrition) is of nutritional significance in many clinical conditions in which amino acids (e.g., tyrosine, cysteine, and glutamine) need to be provided but cannot easily be given in an oral/enteral capacity because they are insoluble or unstable in their free form. The ability to provide these insoluble or unstable amino acids in peptide form that can be used by tissue allows nutrients to be provided in situations in which traditional free amino acid parenteral mixtures are ineffective.

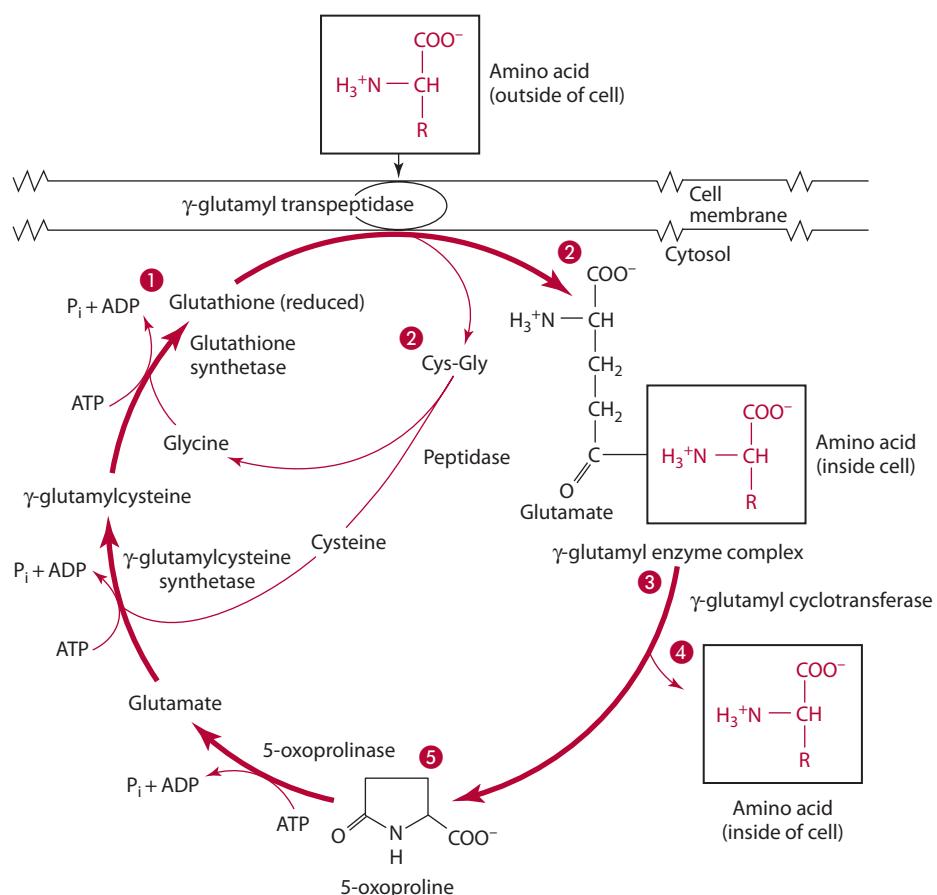
Peptides are thought to be hydrolyzed by peptidases or proteases in the plasma, at the cell membrane (especially the liver, kidney, and muscle), or intracellularly in the cytosol or various organelles following transport as intact peptides [10,18,19]. Peptide transport in renal tubular cells, for example, has been demonstrated and is influenced by molecular structure and the **lipophilicity** (hydrophobicity)

of the amino acids at both the amino (N-) and the carboxy (C-)terminal of the peptide [20,21]. Peptides with either basic or acidic amino acids at either the N- or C-terminal have lower affinity for transport than peptides with neutrally charged side chains at these positions.

Amino acid transport into liver cells (hepatocytes) occurs by some carrier systems similar to those within the intestinal basolateral membrane. The sodium-dependent N system is especially prominent in the periportal cells of the liver and functions as an antiporter to take up sodium and glutamine in exchange for H⁺. The process occurs in reverse in the perivenous hepatic cells; glutamine is released in exchange for H⁺. Hormones and cytokines, such as interleukin-1 and tumor necrosis factor α , influence amino acid transport. System A in hepatocytes, for example, is induced by glucagon [5] and provides amino acid substrates for gluconeogenesis. System GLY is sodium-dependent and specific for glycine; two sodium ions are transported for each glycine. Extrahepatic tissues such as the kidneys also are thought to transport amino

acids by systems similar to those described for the intestinal basolateral membrane. However, an additional system, the γ -glutamyl cycle, is thought to be important in transporting amino acids through membranes of renal tubular cells, erythrocytes, and perhaps neurons.

In the γ -glutamyl cycle, glutathione acts as a carrier of selected neutral amino acids into cells. Glutathione, synthesized and found in most cells of the body, is a thiol and tripeptide consisting of glycine, cysteine, and glutamate. Intracellular availability of cysteine is thought to be a major influence on glutathione's synthesis within cells. As shown in Figure 6.10, an unusual peptide linkage occurs in glutathione, between the γ -carboxyl group of glutamate and the α -amino group of cysteine. In the γ -glutamyl cycle (Figure 6.11), glutathione in its reduced form reacts with γ -glutamyl transpeptidase located in cell membranes, forming a γ -glutamyl enzyme complex. The glutamate part of the glutathione molecule remains with the enzyme complex, whereas cysteinylglycine is released into the cell cytoplasm and eventually cleaved into its constituent amino acids by a



- ① Glutathione reacts with γ -glutamyl transpeptidase to form a γ -glutamyl enzyme complex.
- ② The glutamate portion of glutathione remains attached to the enzyme complex while cysteinylglycine is released and an amino acid binds to the glutamate enzyme complex.
- ③ γ -glutamyl cyclotransferase cleaves the peptide bond between the amino acid and the γ -carbon of the glutamate enzyme complex.
- ④ The free amino acid can be used within the cell.
- ⑤ 5-Oxoprolinase generated from step 3 is used to reform glutamate and via several steps glutathione (step 1).

Figure 6.11 The γ -glutamyl cycle for transport of amino acids.

cytosolic peptidase. The γ -glutamyl enzyme complex functions by binding to a neutral amino acid at the cell surface and carrying it by way of a γ -carboxyl peptide linkage into the cell. Within the cell, γ -glutamyl cyclotransferase can cleave the peptide bond between the neutral amino acid and the γ -carbon of glutamate. Glutathione is resynthesized within the cell from cysteine, glutamate, and glycine in a series of energy-dependent reactions. The neutral amino acid that has just been released within the cell may help synthesize new proteins or nitrogen-containing molecules, or it may be catabolized.

Amino Acid Metabolism

The liver is the primary site for the uptake of most amino acids (about 50%–65%) following ingestion of a meal. The liver is thought to monitor the absorbed amino acids and to adjust the rate of their metabolism (including catabolism, or breakdown of amino acids, and anabolism, or use of amino acids for synthesis) according to the needs of the body. Typically, of the amino acids entering the liver after a meal, about 20% are used to synthesize proteins and nitrogen-containing compounds; of this 20% of amino acids used for synthesis, most of what is synthesized remains in the liver, and the rest is released into the plasma. Each of these areas—the synthesis of proteins, nitrogen-containing nonprotein compounds, and purine and pyrimidine bases; protein synthesis overview; amino acid catabolism overview; and the hepatic catabolism and use of amino acids—is addressed in this section.

SYNTHESIS OF PLASMA PROTEINS, NITROGEN-CONTAINING NONPROTEIN COMPOUNDS, AND PURINE AND PYRIMIDINE BASES

The liver cells, like other body cells, use the amino acids received from portal blood and general circulation to synthesize protein. Many of the proteins, such as enzymes, that are made in the liver remain in the liver; other proteins, however, are released into the plasma. The concentration of total protein in human plasma typically ranges up to about 7.5 g/dL. The proteins found in plasma consist of primarily glycoproteins but also include simple proteins and lipoproteins. These plasma proteins perform a variety of functions. A few of the hundreds of plasma proteins are discussed in the next section.

Plasma Proteins

Albumin, the most abundant of the plasma proteins, is synthesized by the liver and released into the blood. A healthy person makes about 9 to 12 g albumin per day [22]. Changes in osmotic pressure and osmolality of

extracellular spaces affect the rate of albumin synthesis in the body. Albumin in the plasma maintains oncotic pressure and transports nutrients such as vitamin B₆; minerals including zinc, calcium, and small amounts of copper; nutrients such as fatty acids; and the amino acid tryptophan. Some drugs and hormones are also transported by albumin. Albumin is used with a few other proteins in the blood to assess an individual's protein status, specifically visceral (internal organ) protein status. Because of albumin's relatively long half-life (~14–18 days), however, it is not as good or as sensitive an indicator of visceral protein status as some of the other plasma proteins. The half-life is the time it takes for 50% of the amount of a protein such as albumin (or nonprotein compound) to be degraded. Albumin is degraded primarily in vascular endothelial cells.

Two other proteins synthesized by the liver and released into plasma are transthyretin (also called prealbumin) and retinol-binding protein (which is complexed together and involved with retinol, or vitamin A, and thyroid hormone transport). Transthyretin and retinol-binding protein, like albumin, are used as biochemical indicators of visceral protein status. Because transthyretin and retinol-binding protein have relatively shorter half-lives (~2 days and 12 hours, respectively) than albumin, they are more sensitive indicators of changes in visceral protein status than is albumin. The concentrations of albumin, prealbumin, and retinol-binding protein diminish in the blood over varying time lengths (depending on their half-life) in people, for example, who have ingested inadequate dietary protein because of illness. Typically, plasma concentrations of albumin <3.5 g/dL, prealbumin (transthyretin) <18 mg/dL, and retinol-binding protein <2.1 mg/dL suggest inadequate visceral protein status in an individual. Such people need a diet high in energy (kcal) and protein to promote improvements in status (assuming the liver is healthy).

Some of the other proteins made by the liver and released into the blood are those needed for blood clotting, for immunoprotection, and for nutrient transport. Many immunoproteins and transport proteins are globulins, of which several classes exist:

- α 1-globulins: glycoproteins, high-density lipoproteins (for lipid transport)
- α 2-globulins: glycoproteins, haptoglobin (for free hemoglobin transport), ceruloplasmin (for copper transport and oxidase activity), prothrombin (for blood coagulation), and very low density lipoproteins (for lipid transport)
- β -globulins: transferrin (for iron and other mineral transport) and low-density lipoproteins (for lipid transport)
- γ -globulins: immunoglobulins or antibodies (for immunoprotection)

Another group of proteins synthesized in the liver and released in large quantities into the blood as part

of the systemic inflammatory response syndrome to infection (sepsis), injury, or inflammation is called acute phase proteins or positive acute phase reactant proteins. Some examples of these acute phase proteins are C-reactive protein, fibronectin, orosomucoid (also called α 1 acid glycoprotein), α 1 antitrypsin, haptoglobin, α 2 macroglobulin, ceruloplasmin, metallothionein, and serum amyloid A. Collectively, these proteins perform a variety of functions that protect the body, such as stimulating the immune system, promoting wound healing, and chelating and removing free iron from circulation to prevent its use by bacteria for growth. C-reactive protein is used clinically to evaluate inflammation in patients. The concentration of this protein rises dramatically within a few hours of stress and inflammation. Diminishing concentrations of the protein suggest the possibility of a less catabolic state. The Perspective at the end of this chapter provides a more detailed discussion of some of the functions of these acute phase proteins.

The body also generates another group of proteins, called stress or (heat) shock proteins (abbreviated *hsp*). These proteins are synthesized in response to stress, including heat stress and oxidative stress. Exercise or other physical activity in warm environments, among other conditions, promotes the synthesis of these proteins. While the proteins are categorized by molecular weight (e.g., *hsp* 60, *hsp* 70, *hsp* 90, etc.), their functions remain unclear. Some heat shock proteins are thought to facilitate protein folding (that is the formation of the secondary and tertiary protein structures) as the proteins are synthesized in cells. Another hypothesized role of the heat shock proteins is with the repair of denatured or injured proteins.

Nitrogen-Containing Nonprotein Compounds

Nitrogen-containing nonprotein compounds or molecules, of which several exist, are also synthesized in the liver (and often in other sites) from amino acids and perform a number of functions in the body. Some of these compounds (listed below in Table 6.6) and their functions are described in this section.

Not included in this list are a number of biogenic amines, neurotransmitters/hormones, and neuropeptides

Table 6.6 Sources of Nitrogen for Some Nitrogen-Containing Nonprotein Compounds

Nitrogen-Containing Nonprotein Compound	Constituent Amino Acids
Glutathione	Cysteine, glycine, glutamate
Carnitine	Lysine, methionine
Creatine	Arginine, glycine, methionine
Carnosine	Histidine, β -alanine
Choline	Serine

that are synthesized from amino acids in many glands, tissues, and organs throughout the body. A discussion of these compounds is found in this chapter in the section “Brain and Accessory Tissues.” Some of the compounds also are mentioned in sections that discuss the metabolism of amino acids.

Glutathione Glutathione is a tripeptide synthesized from three amino acids: glycine, cysteine, and glutamate. The synthesis occurs in two steps, both ATP dependent, in which first the γ carboxy group of glutamate is attached to the amino group of cysteine by γ glutamyl cysteine synthetase to form a peptidic γ linkage. Availability of cysteine appears to be the major factor influencing glutathione synthesis. Next, glutathione synthetase creates a peptide bond between the amino group of glycine and the carboxy group of cysteine. Glutathione (Figure 6.10) is referred to as a thiol because it contains a sulphydryl (-SH) group in its reduced form (GSH). Glutathione also can be found in cells in its oxidized form designated as GSSG and can be found (up to about 15%) attached to proteins. Normally, the ratio of GSH to GSSG in cells is >10 to 1; the GSH to GSSG ratio represents an indicator of the cell’s redox state.

Glutathione is synthesized and found in the cytosol of most cells of the body, but small amounts also are found within cell organelles and in the plasma. Glutathione has several functions in the body [23]. It is a major antioxidant with the ability to scavenge free radicals (O_2^\bullet and OH^\bullet), thereby protecting critical cell components. With the enzyme glutathione peroxidase, glutathione also protects cells by reacting with hydrogen peroxides (H_2O_2) and lipid hydroperoxides (LOOHs) before they can cause damage to cells. Glutathione also transports amino acids as part of the γ -glutamyl cycle (Figure 6.11). It participates in the synthesis of leukotriene (LT) C4, which mediates the body’s response to inflammation. Glutathione is also involved in the conversion of prostaglandin H2 to prostaglandins D2 and E2 by endoperoxide isomerase. Glutathione can conjugate with nitric oxide to form an S-nitrosoglutathione adduct.

Glutathione synthesis is sensitive to protein intake and pathological conditions. Hepatic GSH and mucosal and systemic GSH concentrations decline with poor protein intake as well as during inflammation and disease; this decline negatively impacts the body, necessitating strategies to enhance or at least maintain GSH concentrations [24]. Glutathione is discussed in further detail in a discussion of selenium and glutathione peroxidase in Chapter 12.

Carnitine Carnitine, another nitrogen-containing compound (Figure 6.12), is made in the liver from lysine that has been methylated using methyl groups from S-adenosyl methionine (SAM), made from the amino acid methionine. Following lysine methylation, trimethyllysine undergoes hydroxylation at the 3 position to form 3-OH trimethyllysine. Hydroxytrimethyllysine is further metabolized to

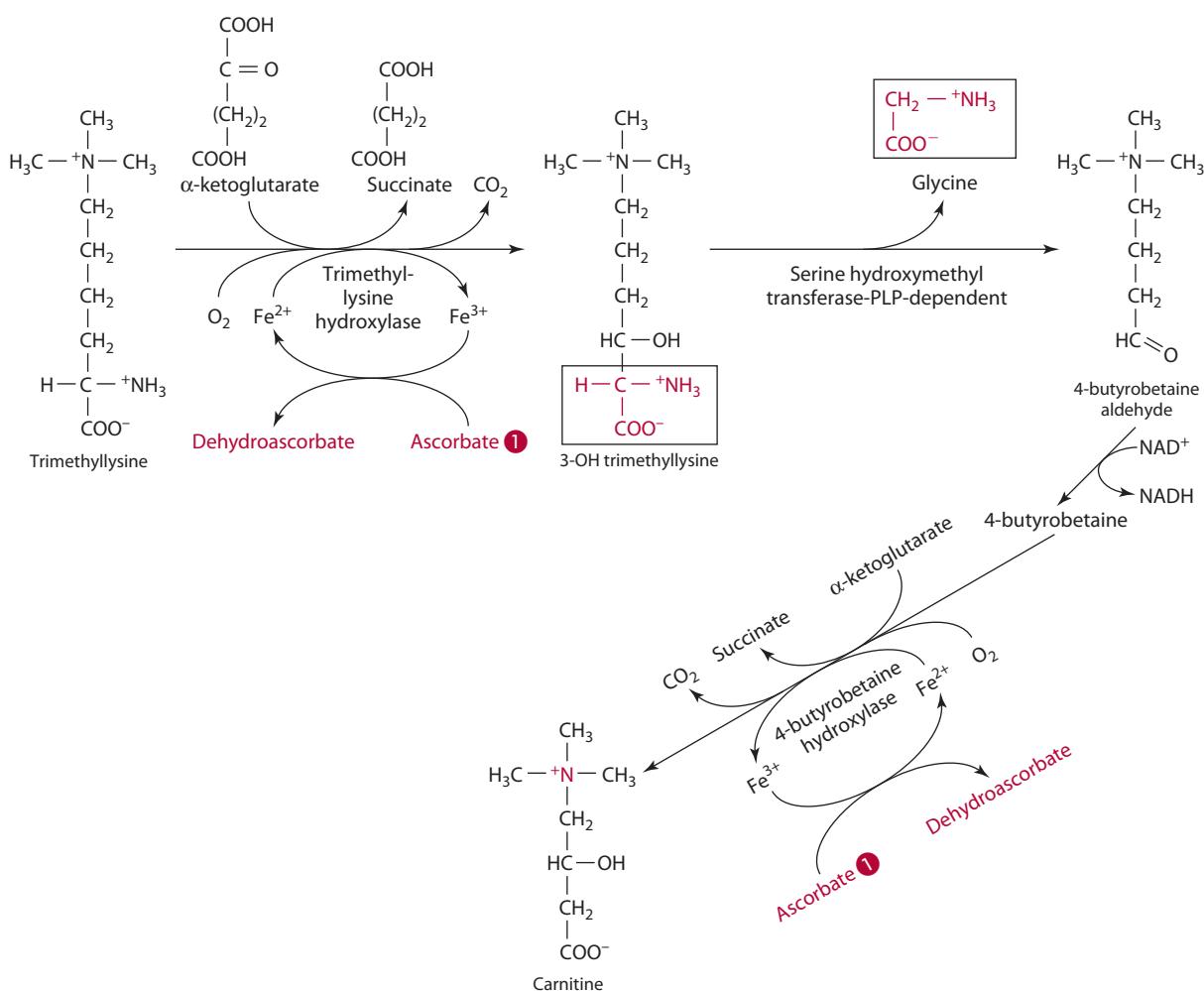


Figure 6.12 Carnitine synthesis.

generate γ -butyrobetaine and subsequently carnitine. Iron, vitamin B_6 (as pyridoxal phosphate-PLP), vitamin C, and niacin participate in the synthesis of carnitine. In addition to being synthesized in the liver and kidney, carnitine is found in foods, especially meats such as beef and pork. In these foods, carnitine may be free or bound (as acylcarnitine) to long- or short-chain fatty acid esters. Carnitine from food is absorbed in the proximal small intestine by sodium-dependent active transport and passive diffusion. Approximately 54% to 87% of carnitine intake is absorbed. Intestinal absorption of carnitine is thought to be saturated with intakes of about 2 g [25]. Muscle represents the primary carnitine pool, although no carnitine is made there. Intramuscular concentrations of carnitine are generally 50 times greater than usual plasma concentrations. Carnitine homeostasis is maintained principally by the kidney, with >90% of filtered carnitine and acylcarnitine being reabsorbed.

- ① Ascorbate functions as a reducing agent in two reactions. In both reactions for carnitine synthesis, the vitamin is needed to reduce the iron atom that has been oxidized (Fe^{3+}) in the reaction back to its reduced (Fe^{2+}) state.

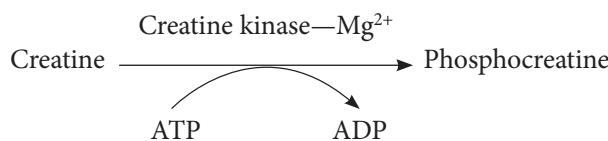
Carnitine, found in most body tissues, is needed for the transport of fatty acids, especially long-chain fatty acids, across the inner mitochondrial membrane for oxidation. The inner mitochondrial membrane is impermeable to long-chain (10) fatty acyl CoAs. This role of carnitine is discussed in more detail in Chapter 5. Carnitine is needed for ketone catabolism for energy. Carnitine also forms acylcarnitines from short-chain acyl CoAs. These acylcarnitines may serve to buffer the free coenzyme (Co) A pool.

Carnitine deficiency, though rare, results in impaired energy metabolism. Advertisements marketing carnitine supplements to help burn fat or supply energy are making false claims. Furthermore, although use of carnitine supplements has been shown to increase plasma and muscle carnitine, studies have not uniformly shown improved physical performance [26–29]. Other studies, however,

have shown beneficial effects of carnitine supplementation in people with a variety of different cardiac problems [25].

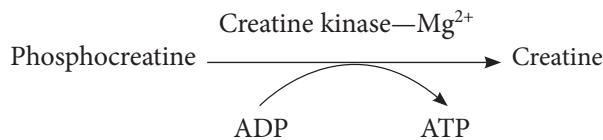
Creatine Creatine (Figure 6.13), a key component of the energy compound creatine phosphate, also called phosphocreatine, can be obtained from foods (primarily meat and fish) or synthesized in the body. The first step in the synthesis of creatine occurs in the kidney, where arginine and glycine react to form guanidoacetate. In this reaction, the guanidinium group of arginine is transferred to glycine; the remainder of the arginine molecule is released as ornithine. The next step in the synthesis of creatine is the methylation of guanidoacetate. This step occurs in the liver using SAM (S-adenosyl methionine) as a methyl donor (see Figure 6.30 and Figure 6.33).

Once synthesized, creatine is released into the blood for transport to tissues. About 95% of body creatine is in muscle, with the remaining 5% in organs such as the kidneys and brain. In tissues, creatine is found both in free form as creatine and in its phosphorylated form. The phosphorylation of creatine to form phosphocreatine is shown here:



Phosphocreatine functions as a “storehouse for high-energy phosphate.” In fact, over half of the creatine in muscle at rest is in the form of phosphocreatine.

Phosphocreatine replenishes ATP in a muscle that is rapidly contracting. Remember, muscle contraction requires energy. This energy is obtained with the hydrolysis of ATP. However, ATP in muscle can suffice for only a fraction of a second. Phosphocreatine, stored in the muscle and possessing a higher phosphate group transfer potential than ATP, can transfer a phosphoryl group to ADP, thereby forming ATP or assisting in ATP regeneration, providing energy for muscular activity. Creatine kinase, also called creatine phosphokinase, catalyzes the phosphate transfer in active muscle as shown here:



The enzyme, abbreviated CK or CPK, is made up of different subunits in different tissues. For example, in the heart,

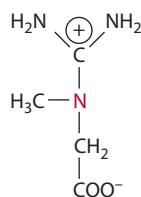


Figure 6.13 Creatine.

creatine kinase is made up of two subunits designated M and B. (The brain and muscle also have creatine kinase but in these tissues the enzyme is made up of the BB and MM subunits, respectively.) Damage to the heart as with a heart attack causes the enzyme to “leak” out of the heart and become present in elevated concentrations in the blood. An elevation in CK-MB in the blood along with other indicators is used to diagnose a heart attack. Damage to skeletal muscle as may occur with trauma results in elevations of CK-MM in the blood.

The availability and use of phosphocreatine by muscle are thought to delay the breakdown of muscle glycogen stores, which upon further catabolism also can be used by muscle for energy. Phosphocreatine and creatine, however, do not remain in the muscle for extended periods of time. Both compounds spontaneously cyclize in a nonreversible, nonenzymatic reaction to form creatinine, which is excreted by the kidneys into the urine. Urinary creatinine excretion is often used as an indicator of somatic muscle mass, as discussed in the “Nitrogen-Containing Compounds as Indicators of Muscle Mass and Muscle/Protein Catabolism” section of this chapter.

Creatine supplements have been shown to increase (~20%–50%) muscle total creatine concentrations and the amount of short-duration maximal exercise (such as sprints and power-type activities separated by intervals of recovery) that can be performed [30–36]. Typical dosages were 5 g creatine monohydrate taken four times per day for a total of 20 g/day; supplements generally were consumed for 5 or 6 days. Ingestion of a carbohydrate solution with creatine supplements resulted in greater muscle creatine accumulation than did ingestion of creatine alone [34,35]. Some short-term positive effects of creatine included reduced decline in peak muscle torque production during repeat bouts of high-intensity isokinetic contractions, higher peak isokinetic torque production sustained during repeat bouts of maximal voluntary contraction, and increased whole-body exercise performance during two initial bouts of maximal isokinetic cycling lasting 30 seconds [32–37]. Other studies, for example among endurance athletes and highly trained swimmers, however, have reported no effects on performance [32,37,38]. Furthermore, side effects associated with long-term use of creatine are unknown.

Carnosine Carnosine (β -alanyl histidine; Figure 6.14) is made in the body from histidine and β -alanine by an energy-dependent reaction catalyzed by carnosine synthetase. In

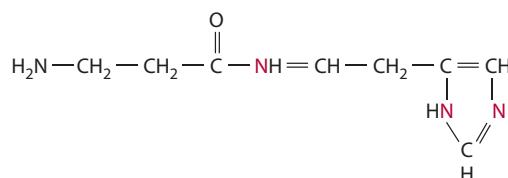


Figure 6.14 Carnosine.

the body, carnosine is found mainly in skeletal muscle, cardiac muscle, and the brain, as well as in the kidneys and stomach. Related compounds include a methylated form of carnosine known as anserine (β -alanyl methylhistidine) and homocarnosine (γ -aminobutyryl histidine), among others. Carnosine also is available in foods, primarily meats, and can be absorbed intact from the intestine by way of peptide transporters. While not all the functions of carnosine have been identified, some studies show carnosine to exhibit antioxidant activity, scavenging hydroxyl and superoxide radicals, quenching singlet oxygen, suppressing lipid peroxidation, and reacting with protein carbonyls [39,40]. In muscle, carnosine may regulate intracellular calcium and contractility [41].

Choline Choline (Figure 6.15) is made in the body from methylation of serine using S-adenosyl methionine (SAM). Choline is also found in foods, in small amounts in free form and more commonly as part of the phospholipid lecithin (phosphatidyl choline). Foods rich in lecithin include eggs, liver and other organ meats, muscle meats, wheat germ, and legumes such as soybeans and peanuts. Lecithin is also added to many foods as an emulsifier.

In the body, choline functions as a methyl donor and as part of the neurotransmitter acetylcholine, the phospholipid phosphatidyl choline (commonly called lecithin), and sphingomyelin. To be converted to acetylcholine, free choline crosses the blood-brain barrier and enters cerebral cells from the plasma through a specific choline transport system. Within the presynaptic terminal of the neuron, acetylcholine is formed by the action of choline acetyltransferase as follows:



Concentrations of choline in cholinergic neurons typically are below the K_m of choline acetyltransferase; thus, the enzyme normally is not saturated. Acetyl CoA is thought to arise from glucose metabolism by neural glycolysis and the action of the pyruvate dehydrogenase complex. Choline also can be recycled—that is, phospholipases can liberate choline from lecithin and sphingomyelin as needed, and acetylcholinesterase can hydrolyze acetylcholine following synaptic transmission.

Choline is oxidized in the liver and kidneys. In the liver, choline oxidation generates betaine, which functions as a methyl donor to regenerate methionine from homocysteine. Further metabolism of betaine (also called trimethylglycine) generates dimethyl glycine (also called sarcosine), which may be catabolized to glycine, methylene tetrahydrofolate, carbon dioxide, and ammonium ion.

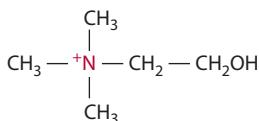


Figure 6.15 Choline.

Experimental diets devoid of choline can result in decreases in plasma choline and phosphatidylcholine concentrations as well as alterations in some liver enzymes. Animals devoid of dietary choline develop a fatty liver accompanied by some hepatic necrosis. The Food and Nutrition Board has suggested that an adequate intake consists of 425 mg and 550 mg choline for adult females and males, respectively [42]. Such intakes are easily obtained through dietary consumption of animal products and foods containing fats. A tolerable upper intake level of 3.5 g choline daily also has been set [42]. The **tolerable upper intake level** represents the highest level of daily intake that is likely to pose no risks of adverse health effects to most people in the general population [42].

Purine and Pyrimidine Bases

Another group of compounds derived in part from amino acids consists of purine and pyrimidine bases. Purine and pyrimidine bases are main constituents of two nucleic acids—deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Remember these nucleic acids are made up of a five carbon sugar, a phosphoric acid, and nitrogenous bases. These nitrogenous bases can be divided into two categories—pyrimidines and purines. The pyrimidines are six-membered rings containing nitrogen atoms in positions 1 and 3. The pyrimidine bases include uracil, cytosine, and thymidine. Deoxycytidine and thymidine (or called deoxythymidine) are found in DNA. Cytidine and uridine are present in RNA. The purines are made up of two fused rings with nitrogen atoms in positions 1, 3, 7, and 9. The purine bases include adenine and guanine and are found in DNA as deoxyadenosine and deoxyguanosine and in RNA as adenosine and guanosine. A brief review of purine and pyrimidine synthesis and degradation follows.

The synthesis of the nitrogen-containing bases used to make nucleic acids and nucleotides occurs for the most part *de novo* in the liver. The individual steps in pyrimidine synthesis are shown in Figure 6.16. First, synthesis of the pyrimidines uracil, cytosine, and thymine (or in nucleotide form UTP, CTP, and TTP respectively) is initiated by the formation of carbamoyl phosphate from glutamine, CO_2 , and ATP. The enzyme carbamoyl phosphate synthetase II catalyzes this reaction in the cytoplasm and is distinct from carbamoyl phosphate synthetase I, which is needed in the initial step of urea synthesis and is found in the mitochondria. Second, carbamoyl phosphate reacts with the amino acid aspartate to form N-carbamoylaspartate. Aspartate transcarbamoylase catalyzes the reaction, which is the committed step in pyrimidine biosynthesis. Following several additional reactions, detailed in Figure 6.16, uridine monophosphate (UMP) is synthesized. Defects in the activity of either OMP decarboxylase used to make UMP (reaction 6, Figure 6.16) or orotate phosphoribosyl transferase (reaction 5, Figure 6.16) cause the genetic disorder orotic aciduria. This condition is characterized by

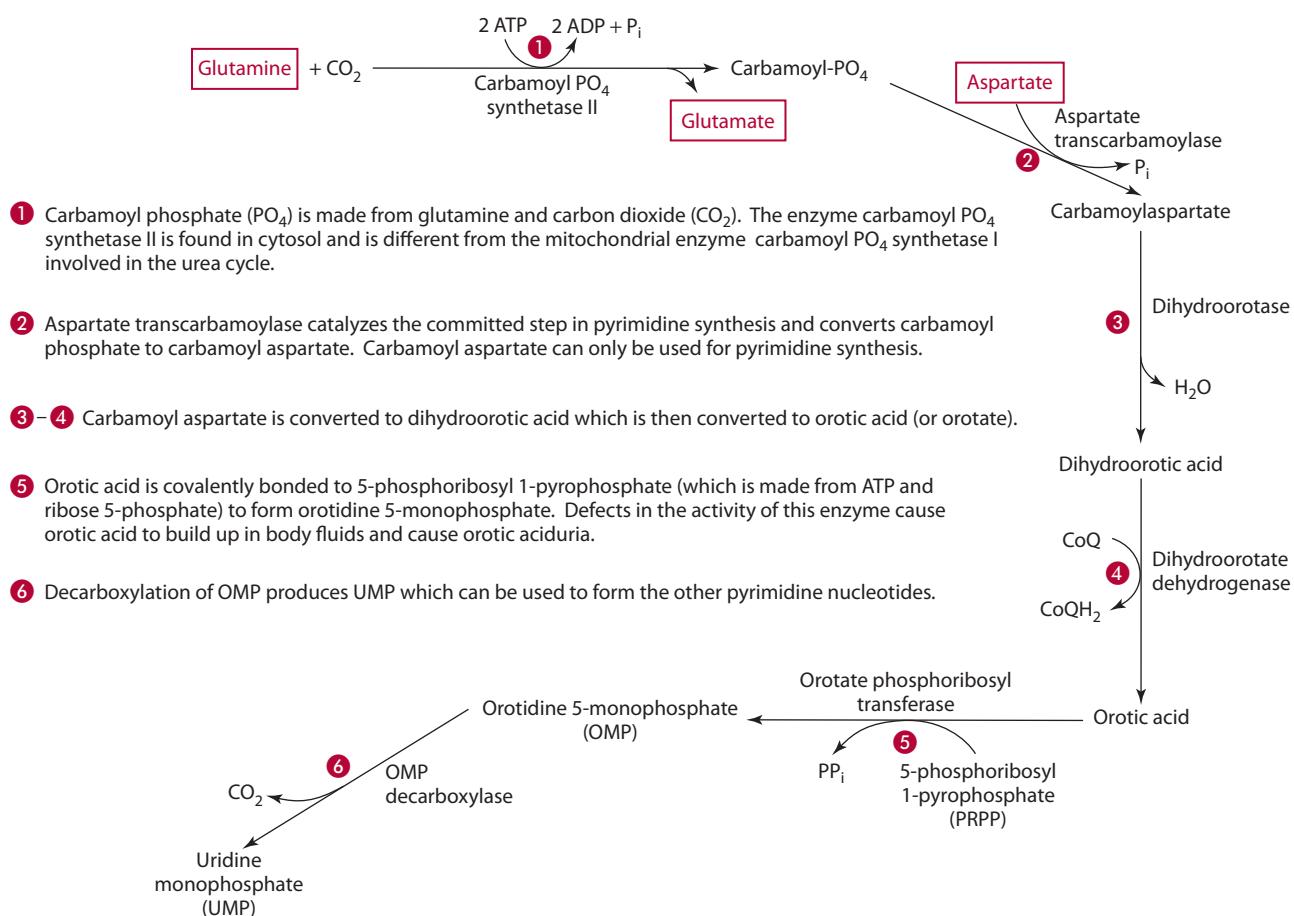


Figure 6.16 The initial reactions of pyrimidine synthesis.

megaloblastic anemia, leukopenia, retarded growth, and the excretion of large amounts of orotic acid in the urine.

The interconversions among the pyrimidines are shown in Figure 6.17 and discussed next. Once uridine monophosphate (UMP) is formed, it may react with other nucleoside di- and triphosphates. UMP can be converted to uridine diphosphate (UDP) utilizing ATP. UDP can be converted to uridine triphosphate (UTP) also using ATP, and UTP can be converted to cytosine triphosphate (CTP) using ATP and an amino group from glutamine. Alternately, UDP can be reduced to deoxy(d)UDP by ribonucleotide reductase; this reaction requires riboflavin as FADH_2 and the protein thioredoxin. DeoxyUDP can then be converted to dUMP. The formation of deoxythymidine (or just called thymidine) monophosphate (dTMP or TMP) from dUMP is catalyzed by thymidylate synthetase; the reaction requires 5,10 methylene tetrahydrofolate and forms dihydrofolate (DHF). Dihydrofolate reductase is needed to convert DHF to tetrahydrofolate, which is then converted to 5,10 methylene tetrahydrofolate and thus allows for dTMP synthesis. DeoxyTMP can be phosphorylated to form deoxythymidine diphosphate (dTDP) and then phosphorylated again to produce deoxythymidine triphosphate (dTTP or abbreviated TTP). Thus, through these reactions CTP, (d)TTP, and UTP have been generated and can be used for the

synthesis of DNA and RNA. The pyrimidine ring structure and its sources of carbon and nitrogen atoms along with the structures of the pyrimidine bases are shown in Figure 6.18. CTP is also used in phospholipid synthesis and UTP is used to form activated intermediates in the metabolism of various sugars. Drugs used to treat cancer often target key enzymes needed for the synthesis of purines or pyrimidines, which are needed by human cells and by cancer cells to grow and multiply. The drug methotrexate for example inhibits dihydrofolate reductase activity and thereby decreases dTMP (and thus TTP) formation. Rapidly dividing cells such as cancer cells are more susceptible to the effects of these drugs.

The purine bases adenine and guanine (Figure 6.18) are synthesized de novo as nucleoside monophosphates by sequential addition of carbons and nitrogens to ribose-5-phosphate that has originated from the hexose monophosphate shunt. As shown in Figure 6.19, in the initial reaction, ribose 5-phosphate reacts with ATP to form 5-phosphoribosyl 1-pyrophosphate (PRPP). Glutamine then donates a nitrogen to form 5-phosphoribosylamine. This step represents the committed step in purine nucleotide synthesis. Next in a series of reactions, nitrogen and carbon atoms from glycine are added, formylation occurs by tetrahydrofolate, another nitrogen atom is donated by

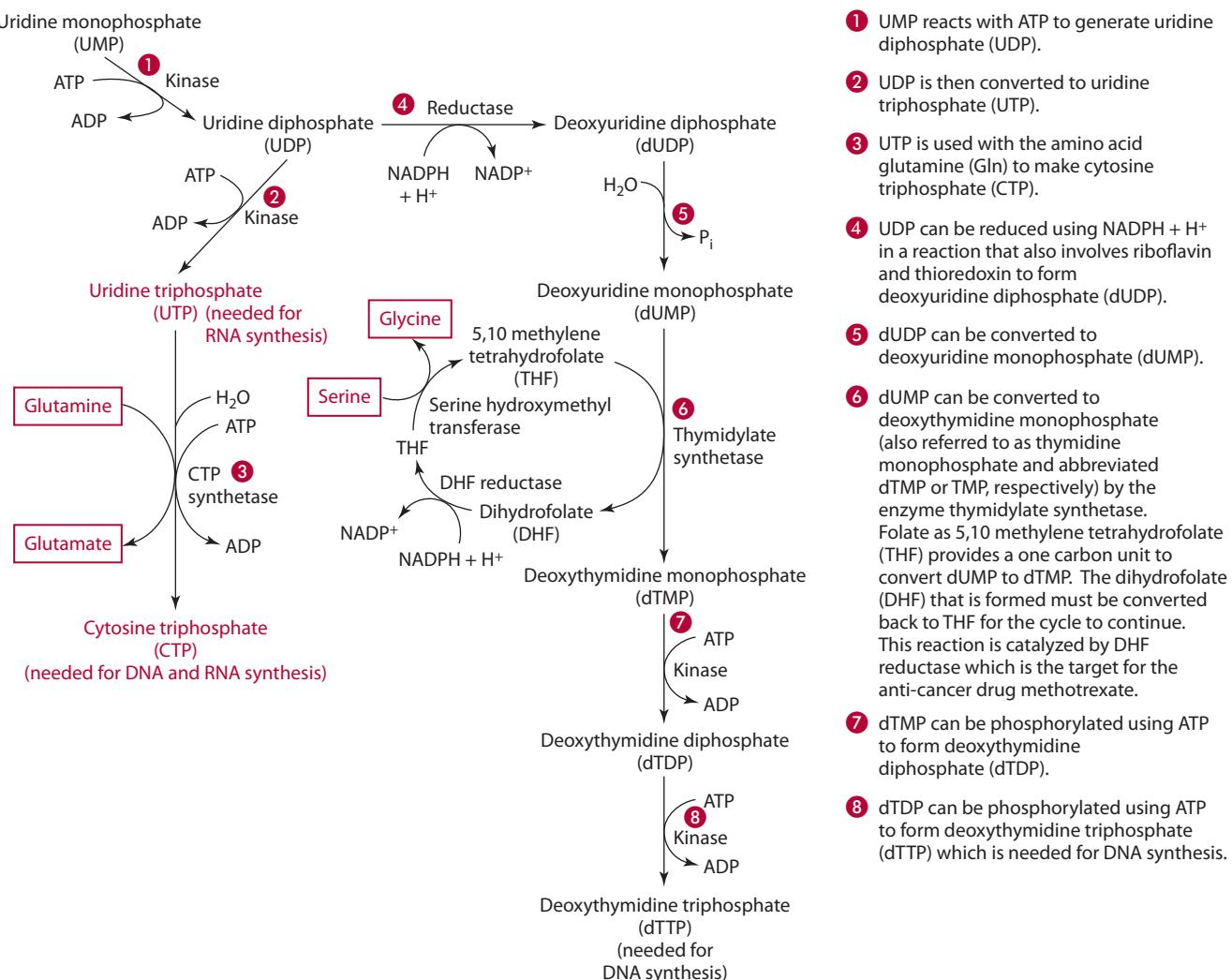


Figure 6.17 The formation of the pyrimidine nucleoside triphosphates UTP, CTP, and TTP for DNA and RNA synthesis.

the amide group of glutamine, and ring closure occurs. Another set of reactions occurs involving the addition of carbons from carbon dioxide and from 10-formyl THF (from folate) and a nitrogen from aspartate. The net result of all of these reactions is the formation of a purine ring (Figure 6.18). The ring is thus derived from components of several amino acids, including glutamine, glycine, and aspartate, as well as from folate and CO₂.

The formation of purines for DNA and RNA synthesis is shown in Figure 6.20. Inosine monophosphate (IMP) is used to synthesize adenosine monophosphate (AMP) and guanosine monophosphate (GMP). AMP and GMP are phosphorylated to ADP and GDP, respectively, by ATP. The deoxyribotides are formed at the diphosphate level by converting ribose to deoxyribose, thereby producing dADP and dGDP. ADP can be phosphorylated to ATP by oxidative phosphorylation; the remaining nucleotides are phosphorylated to their triphosphate form by ATP.

Purine nucleotides also can be synthesized by the salvage pathway, which requires much less energy than de novo synthesis. In the salvage pathway, the purine base

adenine reacts with 5-phosphoribosyl 1-pyrophosphate (PRPP) to form AMP + PP_i in a reaction catalyzed by adenine phosphoribosyl transferase. The purine guanine also can react with PRPP to form GMP + PP_i. Hypoxanthine can react with PRPP to form IMP + PP_i. These last two reactions are catalyzed by hypoxanthine-guanine phosphoribosyl transferase. Defects in this enzyme cause the disorder Lesch-Nylan syndrome, a genetic X-linked condition characterized most notably by self-mutilation, such as the biting off of ones fingers, and premature death. Other symptoms include mental retardation and the accumulation of hypoxanthine, phosphoribosyl pyrophosphate, and uric acid in body fluids.

Degradation of pyrimidines involves the sequential hydrolysis of the nucleoside triphosphates to mononucleotides, nucleosides, and, finally, free bases. This process can be accomplished in most cells by lysosomal enzymes. During catabolism of pyrimidines, the ring is opened with the production of CO₂ and ammonia from the carbamoyl portion of the molecule. The ammonia can be converted to urea and excreted. Malonyl CoA and methylmalonyl CoA,

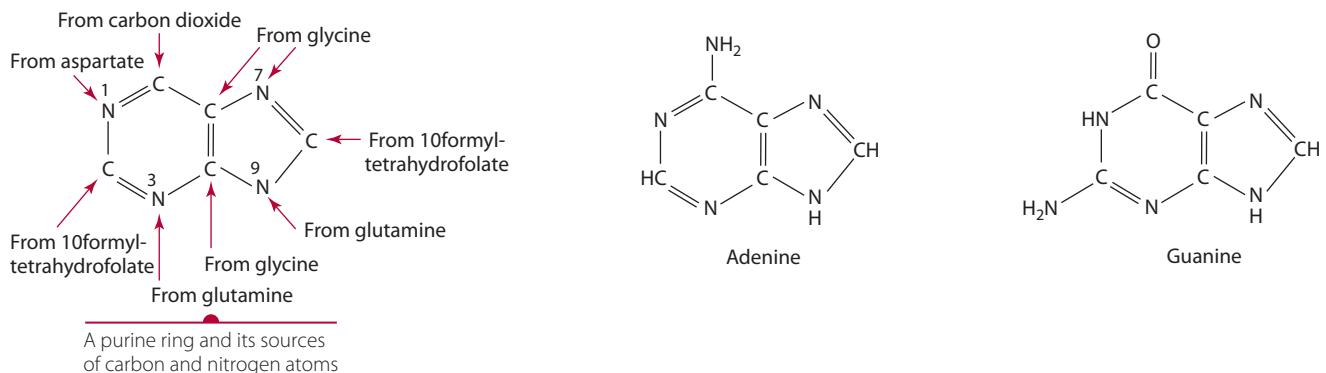
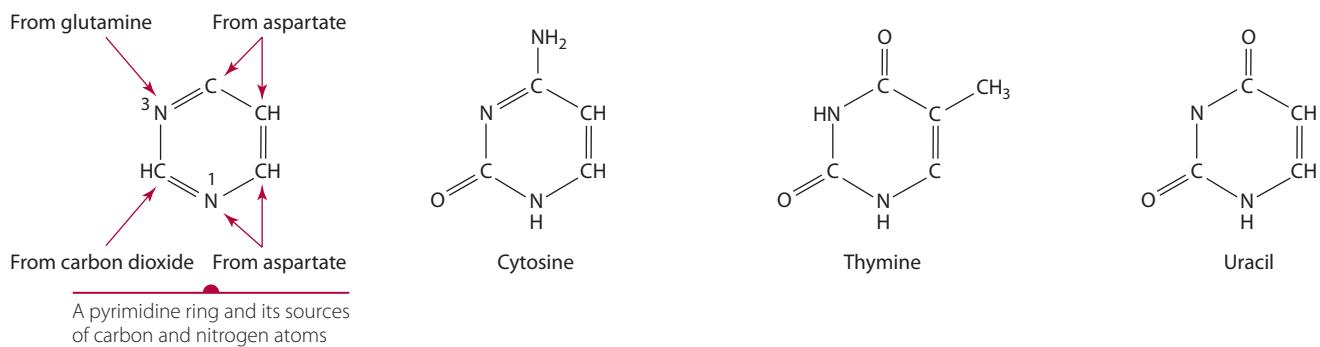


Figure 6.18 The pyrimidine and purine ring structures and the pyrimidine and purine bases. Cytosine, adenine, and guanine are found in both DNA and RNA. Thymine is found in DNA and uracil only in RNA.

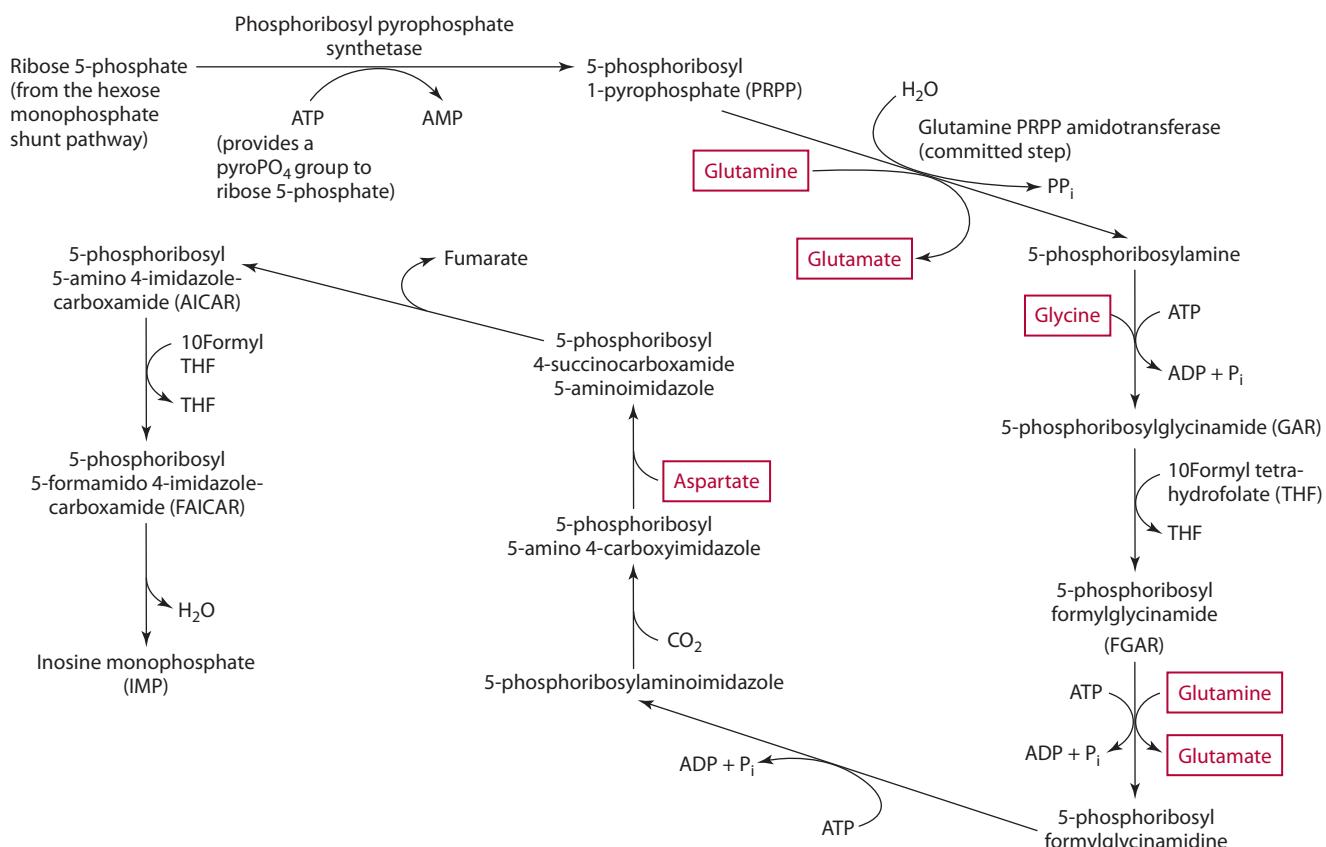


Figure 6.19 Synthesis of inosine monophosphate (IMP), which is used to synthesize other purine nucleotides.

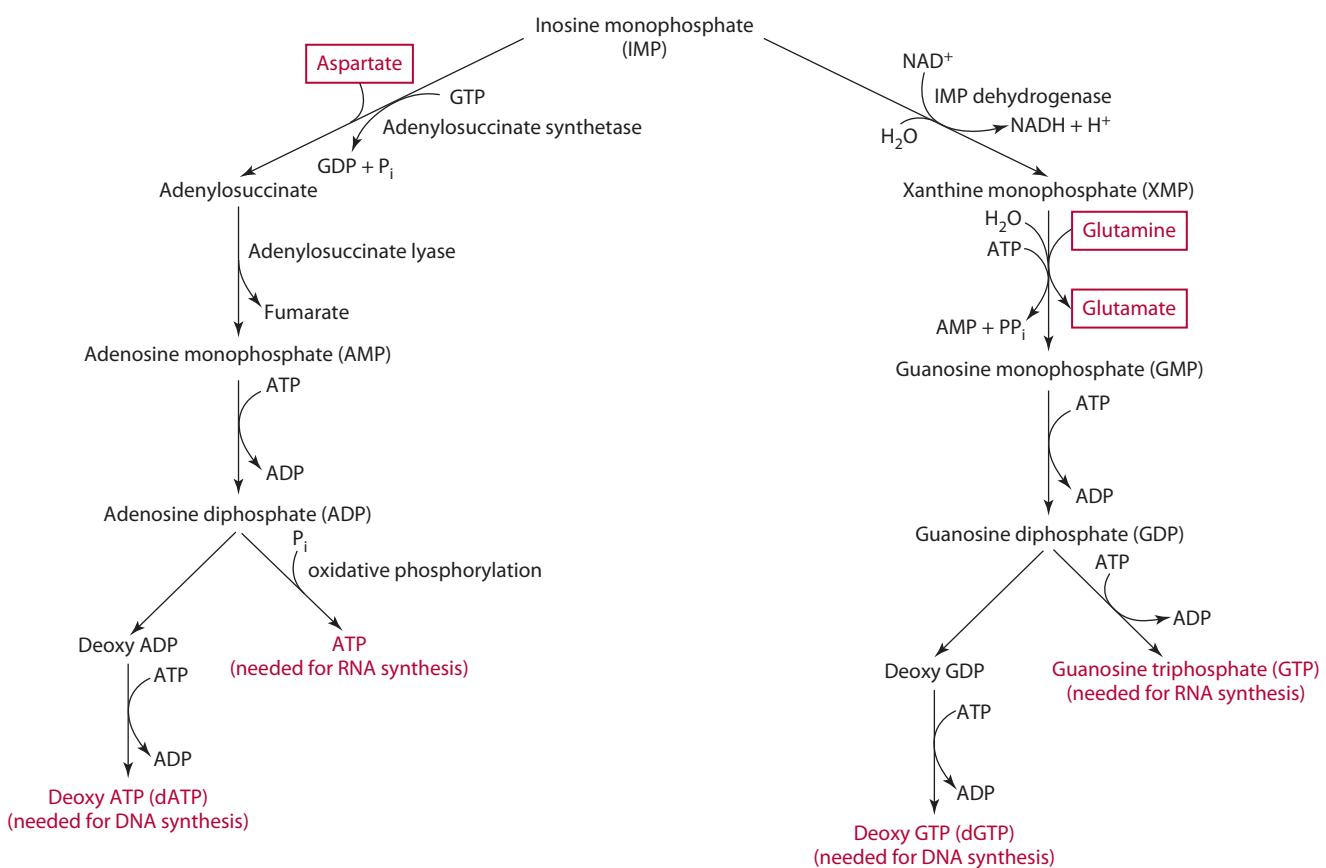


Figure 6.20 The formation of purines needed for DNA and RNA synthesis.

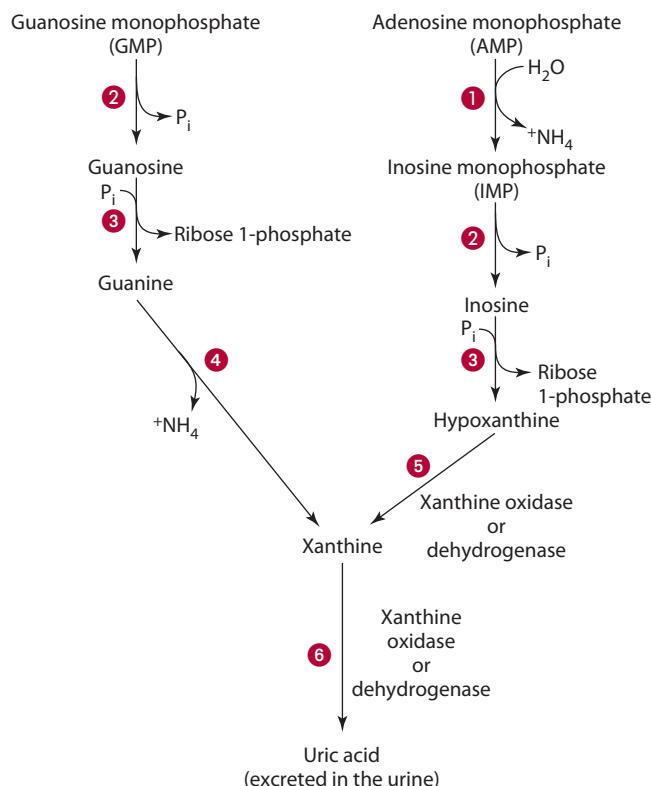
produced from the remainder of the ring, follow their normal metabolic pathways, thus requiring no special excretion route.

Purines (GMP and AMP) are progressively oxidized for degradation primarily in the liver, yielding xanthine, which is converted to uric acid for excretion (Figure 6.21). Xanthine dehydrogenase and oxidase, both molybdenum- and iron-dependent flavoenzymes, convert hypoxanthine (generated from AMP) to xanthine and also convert xanthine (made from both AMP and GMP) to uric acid. Xanthine oxidase uses molecular oxygen and generates hydrogen peroxide while xanthine dehydrogenase uses NAD^+ and forms $\text{NADH} + \text{H}^+$. The uric acid that is produced is normally excreted in the urine, although up to 200 mg also may be secreted into the digestive tract. In the disorder gout and in renal failure, uric acid accumulates in the body causing painful joints among other problems. The drug allopurinol is one of several drugs used to treat gout; it works by binding to the enzyme xanthine oxidase to prevent its interaction with xanthine and hypoxanthine and thus diminish uric acid production. The oxidase (rather than the dehydrogenase) form of the enzyme predominates in several body tissues under conditions of oxygen deprivation (as with a heart attack). The problem is that with oxygen delivery (while restoring the need for oxygen), hydrogen peroxide and free radical production

both increase and may further damage the injured tissues. Research involving introduction of enzymes and antioxidant nutrients to help minimize tissue damage with reoxygenation is ongoing.

PROTEIN SYNTHESIS OVERVIEW

An overview of amino acid use for anabolism is given in Figure 6.22. A summary of the use of selected amino acids for the synthesis of nitrogen-containing nonprotein compounds and selected biogenic amines, hormones, and neuromodulators is depicted in Figure 6.23. Use of amino acids for anabolism occurs throughout the day, but especially following meal ingestion (foods containing carbohydrate, fat, and protein). The amino acids from the diet as well as those generated from degradation of body proteins are metabolized for various roles in various tissues and are used for the synthesis of various body proteins. Insulin secreted in response to carbohydrate (and protein) ingestion promotes cellular uptake and use of the amino acids for protein synthesis. For example, insulin affects (generally stimulates) the transcellular movement of amino acid transporters to the membrane and the activity of several amino acid transporters including, for example, system A, ASC, and N in the liver, muscle, and other tissues. Insulin also antagonizes the activation of some enzymes



- ① AMP is deaminated to produce IMP
- ② IMP and GMP are dephosphorylated generating inosine and guanosine respectively
- ③ A ribose is removed from the inosine and guanosine to form hypoxanthine and guanine respectively
- ④ Guanine is deaminated to form xanthine
- ⑤ Hypoxanthine is converted to xanthine
- ⑥ Xanthine is converted to uric acid which is excreted in the urine

Figure 6.21 The degradation of the purines AMP and GMP generates uric acid.

responsible for amino acid oxidation. The phosphorylation and thus activation of phenylalanine hydroxylase (phenylalanine hydroxylase degrades phenylalanine), for example, is inhibited by insulin. However, should blood glucagon concentrations predominate over insulin as may occur in fasting situations and with untreated diabetes, some amino acids are preferentially used for glucose synthesis (gluconeogenesis). Thus, typically in a healthy person, with eating, protein synthesis increases in the body and degradation of body proteins decreases.

Studies suggest that the rate of protein digestion, however, also can influence protein synthesis. For example, whey protein ingestion caused plasma amino acids to rise more quickly and to higher concentrations, but also to fall more quickly than ingestion of equal amounts of the protein

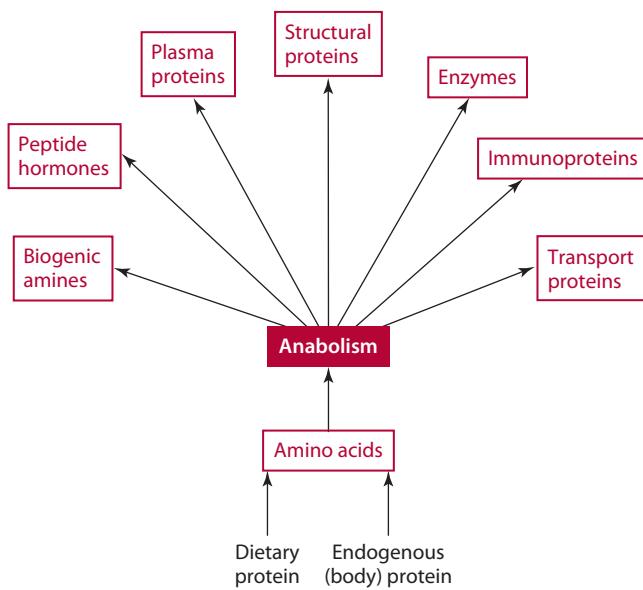


Figure 6.22 Use of amino acids for anabolism. Amino acids are used in a variety of ways in the body.

casein. Whey is thus called a “fast” protein. Fast proteins may be too quickly digested, absorbed, and oxidized to effectively promote protein synthesis. Ingestion of casein (a “slow” protein) resulted in more prolonged and lower plasma amino acid concentrations than ingestion of whey. Further, while ingestion of both proteins stimulated protein synthesis in the body, ingestion of casein (but not whey) inhibited protein degradation by about 30% [43,44]. The inhibition of protein degradation has been attributed to the prolonged hyperaminoacidemia found with casein ingestion. The effects of these “fast” and “slow” digestible proteins on protein synthesis appear to vary. Some studies suggest younger people may better use slow proteins and older people better use fast proteins; however, additional studies are needed [43,44].

The amino acid leucine also appears to play an important role in protein metabolism. Leucine stimulates pancreatic insulin secretion. It also promotes protein synthesis in several tissues including the liver, muscle, and skin through accelerating the initiation and/or elongation phases of mRNA translation. Leucine’s effects are thought to be mediated by intracellular signaling pathways [45–48]. More specifically, leucine is thought to promote protein synthesis through a signaling cascade that in turn stimulates mammalian target of rapamycin (mTOR) causing changes in phosphorylation and ultimately initiation of mRNA translation [45–48]. Mammalian target of rapamycin is thought to integrate information from primarily intracellular amino acid sensing and the insulin mediated signal cascade to initiate translation. Other amino acids are thought to promote changes in cell volume and promote protein synthesis through other intracellular signaling pathways (such as PHAS-1 and p70S 6-kinase) [46,49,50]. Insulin, while promoting protein synthesis, also inhibits

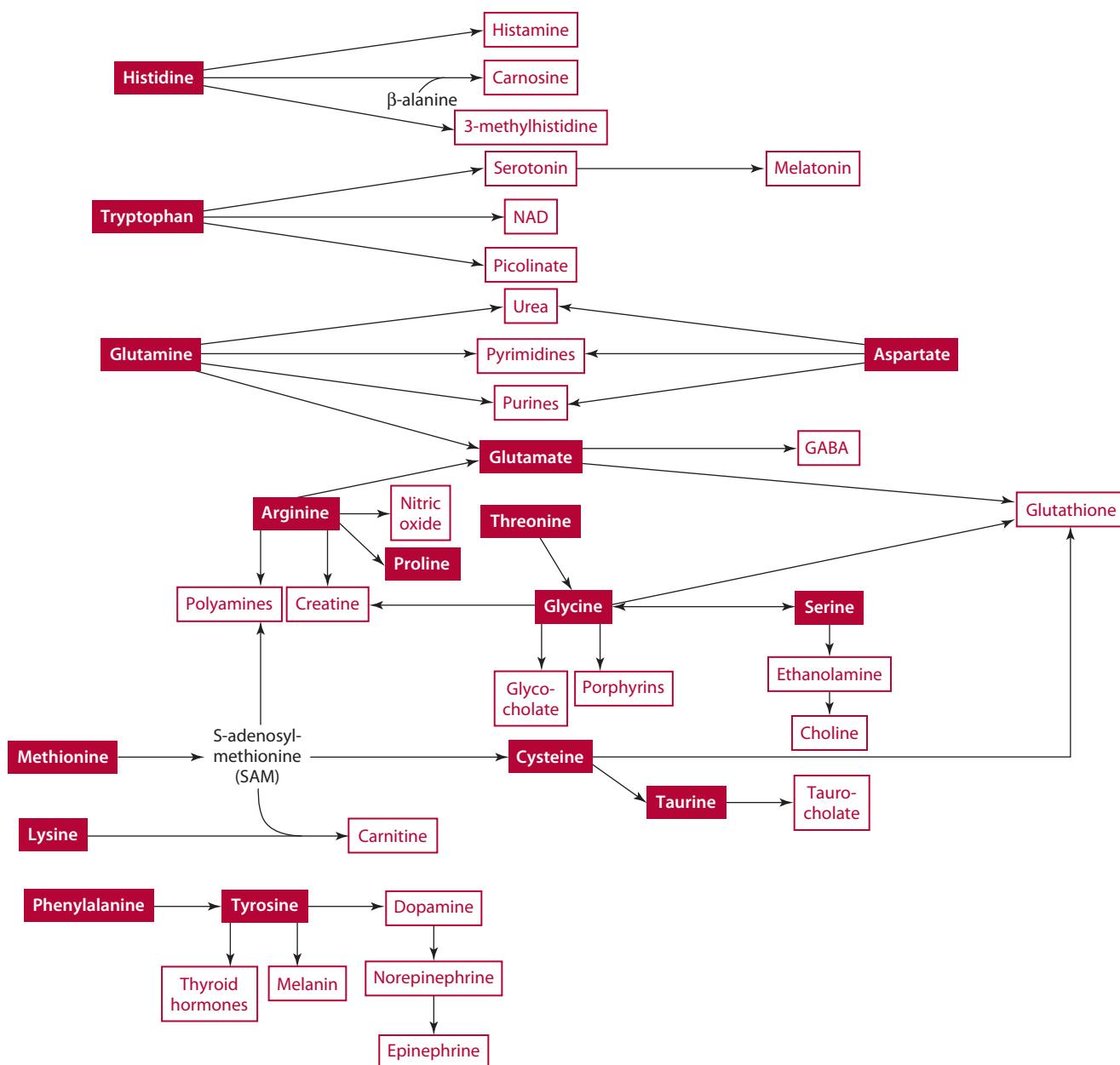


Figure 6.23 A summary of the uses of selected amino acids for the synthesis of nitrogen-containing compounds and selected biogenic amines, hormones, and neuromodulators.

protein degradation. Insulin, for example, has been shown to inhibit the initial steps in ubiquitin-dependent protein degradation (see the section “Cellular Protein Degradation Systems”).

Although protein synthesis typically predominates over protein degradation after eating, the opposite becomes true when food is not eaten. During prolonged periods in which food is not eaten, such as during the overnight hours or a fast, protein synthesis still occurs but at a much lower rate, and protein degradation increases. The tissue that experiences the most protein degradation during these postabsorptive periods is the skeletal muscle. The degradative process is stimulated by cortisol release and by the higher glucagon to insulin ratio in the blood. Amino acids

generated from the degradation of protein can be further catabolized for various uses by the body, as discussed in the next section.

AMINO ACID CATABOLISM OVERVIEW

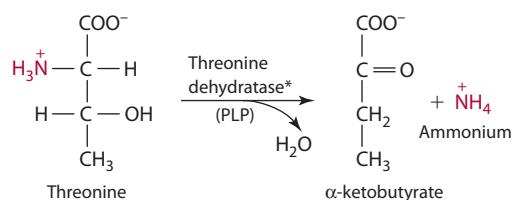
Liver cells have a high capacity for the uptake and catabolism of amino acids. Catabolism of amino acids occurs to varying degrees in different tissues both during fasting periods and after eating. In fact, after a meal, the liver takes up about 50% to 65% of amino acids from portal blood. The liver is the main site for the catabolism of indispensable amino acids, with the exception of the branched-chain amino acids. The rate of hepatic catabolism for the amino

acids, however, differs. Branched-chain amino acids, for example, are catabolized much more slowly in the liver than in muscle. Furthermore, not all amino acids are catabolized in the same regions of the liver. Periportal hepatocytes, for example, catabolize most amino acids with the exception of glutamate and aspartate, which are metabolized by perivenous hepatocytes. The liver derives up to 50% of its energy (ATP) from amino acid oxidation; the energy generated in turn may be used for gluconeogenesis or urea synthesis, among other needs, depending on the body's state of nutriture. This section on amino acid catabolism first focuses on the reactions that occur as amino acids are broken down in liver cells, including first the transamination and/or deamination of amino acids and then the urea cycle. It next discusses the uses of the carbon skeleton of amino acids.

Transamination and/or Deamination of Amino Acids

Usually, the first step in the metabolism of amino acids not used for the synthesis of proteins or nitrogen-containing compounds is the removal of the amino group from the amino acid. Amino acids can undergo deamination and/or transamination to remove amino groups. Deamination reactions involve only the removal of an amino group, with no direct transfer to another compound. Some amino acids that are more commonly deaminated include glutamate, histidine, serine, glycine, and threonine; however, many of these same amino acids also can be transaminated. The enzymes carrying out the deamination reactions are generally lyases, dehydratases, or dehydrogenases. Figure 6.24 shows the deamination of the amino acid threonine by threonine dehydratase to form α -ketobutyrate (another α -keto acid) and ammonia/ammonium. Ammonia is readily used by periportal hepatocytes for urea synthesis. The synthesis of urea in the liver is addressed in the next subsection, "Disposal of Ammonia—The Urea Cycle."

Transamination reactions involve the transfer of an amino group from one amino acid to an amino acid carbon skeleton or α -keto acid (an amino acid without an amino group). The carbon skeleton/ α -keto acid that gains the amino group becomes an amino acid, and the



*The enzyme is called *dehydratase* rather than *deaminase* because the reaction proceeds by loss of elements of water. In the deamination, the amino group from the amino acid is removed. Vitamin B_6 as PLP is required by the enzyme.

Figure 6.24 The deamination of the amino acid threonine. In the deamination, the amino group from the amino acid is removed.

amino acid that loses its amino group becomes an α -keto acid. These reactions are important for the synthesis of many of the body's dispensable amino acids. Transamination reactions are catalyzed by enzymes called aminotransferases. These enzymes typically require vitamin B_6 in its coenzyme form, pyridoxal phosphate (PLP). Some examples of aminotransferases include tyrosine aminotransferase, branched-chain aminotransferases, alanine aminotransferase (ALT; formerly called glutamate pyruvate transaminase and abbreviated GPT), and aspartate aminotransferase (AST; formerly called glutamate oxaloacetate transaminase and abbreviated GOT). These latter two aminotransferases (ALT and AST) are among the most active of the aminotransferases and involve three key amino acids: alanine, glutamate, and aspartate.

Aminotransferases are found in varying concentrations in different tissues. For example, AST is found in higher concentrations in the heart than in the liver, muscle, and other tissues. In contrast, ALT is found in higher concentrations in the liver than in the heart but is also found in moderate amounts in the kidney and small amounts in other tissues. Normal serum concentrations of these enzymes are low; however, with trauma or disease to an organ, serum enzyme concentrations rise and serve as an indicator of both which organ has been damaged and the severity of the organ damage. Thus, with liver damage, one sees higher than normal blood concentrations of AST and ALT as well as other enzymes normally found in the liver such as alkaline phosphatase and lactate dehydrogenase. With heart damage (as may occur with a heart attack), enzymes normally found in the heart "leak" out into the blood because of the cell damage. Heart damage is usually indicated by high blood concentrations of AST and a specific MB form of creatine kinase (also called creatine phosphokinase).

Reactions catalyzed by ALT and AST are shown in Figure 6.25. ALT transfers amino groups from alanine to an α -keto acid (e.g., α -ketoglutarate), forming pyruvate and another amino acid (e.g., glutamate), respectively. AST transfers amino groups from aspartate also to an α -keto acid (e.g., α -ketoglutarate), yielding oxaloacetate and another amino acid (e.g., glutamate), respectively.

These reactions are reversible. Because glutamate and α -ketoglutarate readily transfer and/or accept amino groups, these compounds play central roles in amino acid metabolism.

In summary, the first step in the use of amino acids, for functions other than the synthesis of proteins or nitrogen-containing compounds, requires either transamination or deamination. Transamination reactions can generate dispensable amino acids from indispensable amino acids or create one dispensable amino acid from another dispensable amino acid. The only exceptions are lysine, histidine, and threonine, which do not participate in such reactions. Ammonia generated from oxidative deamination reactions must be safely removed from the system; this is

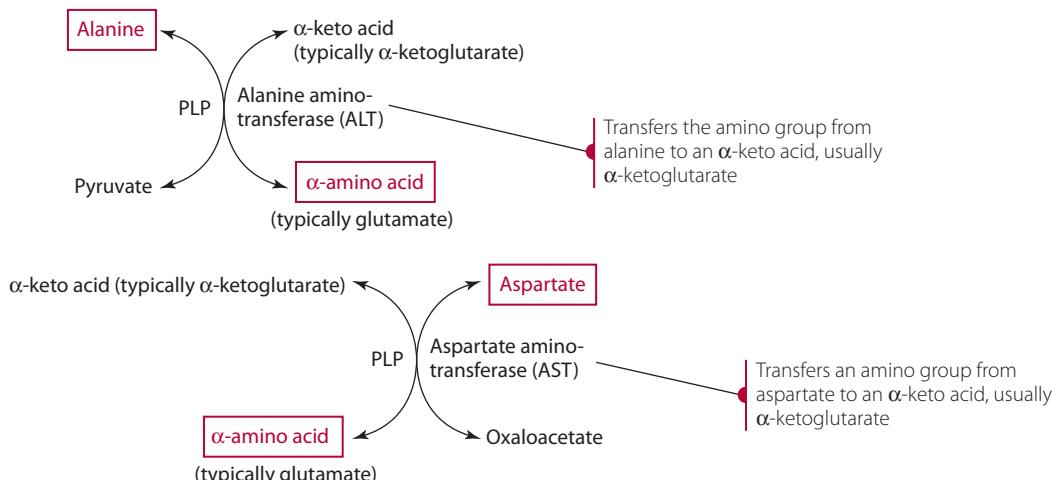


Figure 6.25 Transamination reactions.

accomplished by the actions of the urea cycle, which is discussed next.

Disposal of Ammonia—The Urea Cycle

The urea cycle, discovered by Sir Hans Krebs, functions in the liver and is important for the removal of ammonia from the body. Too much ammonia in the body (as can occur with liver failure) is toxic and can lead to brain malfunction and coma. Some of the sources of ammonia in the body include:

- ammonia formed in the body from chemical reactions such as deamination
- ammonia ingested and absorbed from the foods we eat
- ammonia generated in the gastrointestinal tract from bacterial lysis of urea and amino acids and subsequently absorbed through the enterocyte into the body

The liver has two systems in place to deal with ammonia. First and foremost, periportal hepatocytes are active in ureagenesis. Ammonia from the diet or from intestinal bacterial synthesis enters portal blood and first comes in contact with hepatocytes, specifically, periportal hepatocytes capable of urea synthesis. These same periportal cells are responsible for almost all amino acid catabolism, so ammonia generated during amino acid degradative reactions can be immediately taken up for urea synthesis. However, should the periportal cells fail to use all the ammonia, a second group of hepatocytes, the perivenous hepatocytes, are capable of utilizing the ammonia for glutamine synthesis. The perivenous cells thus provide a “backup” system for ammonia that escaped involvement in urea production.

Figure 6.26 reviews key compounds of the urea cycle and shows its relationship with amino acids and the TCA cycle. The reactions of the urea cycle are broken down in the following list:

- Ammonia (NH_3) combines with CO_2 or HCO_3^- to form carbamoyl phosphate in a reaction catalyzed by mitochondrial carbamoyl phosphate synthetase I (CPSI) and using 2 mol of ATP and Mg^{2+} . N-acetyl-glutamate

(NAG), made in the liver and intestine, is required as an allosteric activator to allow ATP binding.

- Carbamoyl phosphate reacts with ornithine in the mitochondria, using the enzyme ornithine transcarbamoylase (OTC) to form citrulline. Citrulline in turn inhibits OTC activity.
- Aspartate reacts with citrulline once it has been transported into the cytoplasm (cytosol). This step, catalyzed by argininosuccinate synthetase, is the rate-limiting step of the cycle. ATP (two high-energy bonds) and Mg^{2+} are required for the reaction, and argininosuccinate is formed. Argininosuccinate, arginine, and $\text{AMP} + \text{PP}_i$ inhibit the enzyme.
- Argininosuccinate is cleaved by argininosuccinase in the cytoplasm to form fumarate and arginine. Both fumarate and arginine inhibit argininosuccinase activity. Argininosuccinase is found in a variety of tissues throughout the body, especially the liver and kidneys. High concentrations of arginine increase the synthesis of N-acetylglutamate (NAG), which is needed for the synthesis of carbamoyl phosphate in the mitochondria.
- Urea is formed and ornithine is re-formed from the cleavage of arginine by arginase I, a manganese requiring hepatic enzyme. Arginase activity is inhibited by both ornithine and lysine and may become rate limiting under conditions that limit manganese availability or that alter its affinity for manganese [17,51].

Overall, the urea cycle uses four high-energy bonds. Oxidations in the TCA cycle coupled with phosphorylation through the electron transport chain can provide the ATP required for urea synthesis. The urea molecule derives one nitrogen from ammonia, a second nitrogen from aspartate, and its carbon from $\text{CO}_2/\text{HCO}_3^-$. Once formed, urea typically travels in the blood to the kidneys for excretion in the urine; however, up to about 25% of urea may be secreted from the blood into the intestinal lumen, where it may be degraded by bacteria to yield ammonia.

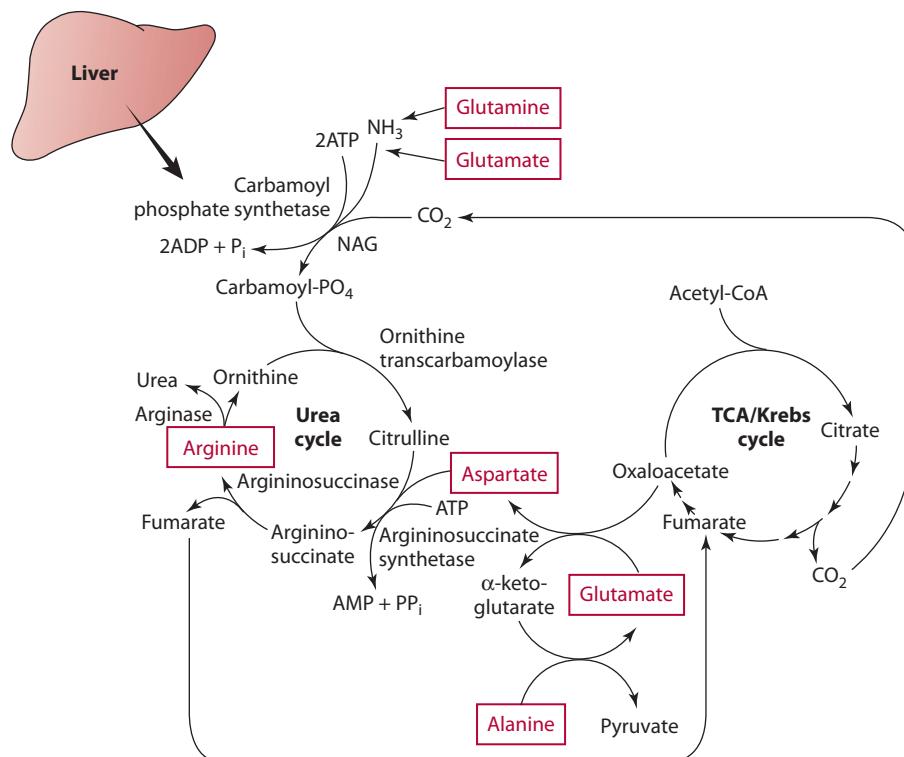


Figure 6.26 Interrelationships of amino acids and the urea and TCA/Krebs cycles in the liver. The individual reactions in the urea cycle are discussed in the text.

Activities of urea cycle enzymes fluctuate with diet and hormone concentrations. For example, with low protein diets or acidosis, urea synthesis (the amount of mRNA for each of the enzymes) diminishes and urinary urea nitrogen excretion decreases significantly. Thus, substrate availability results in short-term changes in the rate of ureagenesis. In the healthy individual with a normal protein intake, blood urea nitrogen (BUN) concentrations range from about 8 to 20 mg/dL, and urinary urea nitrogen represents about 80% of total urinary nitrogen. Glucocorticoids and glucagon, which promote amino acid degradation, typically increase mRNA for the urea cycle enzymes and promote amino acid degradation [51].

Several urea cycle enzyme-deficient disorders have been characterized. Defects in any one of the enzymes of the urea cycle are possible. Urea cycle enzyme defects typically result in high levels of blood ammonia (hyperammonemia) and necessitate a nitrogen-restricted diet, which may be coupled with supplements of carnitine or single amino acids, among other compounds.

In those with advanced liver disease, urea synthesis is diminished and blood ammonia concentrations increase. This rise is thought to contribute to hepatic encephalopathy. Medical treatment for encephalopathy aims at decreasing blood ammonia concentrations. Drugs such as lactulose are given to acidify the gastrointestinal tract contents and promote the diffusion of the ammonia out of the blood and into the gastrointestinal tract. A very low protein diet is prescribed. Further, antibiotics are prescribed that promote the destruction of intestinal tract bacteria that generate ammonia.

An Overview of Metabolism of the Carbon Skeleton/α-Keto Acid

Once an amino group has been removed from an amino acid, the remaining molecule is called a carbon skeleton or α-keto acid.



Carbon skeletons of amino acids can be further metabolized with the potential for multiple uses in the cell. An amino acid's carbon skeleton, for example, can be used to produce:

- energy
- glucose
- ketone bodies
- cholesterol
- fatty acids

The potential use of the carbon skeleton depends in part on the original amino acid from which it was derived. Whereas all amino acids can be completely oxidized to generate energy, not all amino acids can be used for synthesis of glucose. Furthermore, the fate of the amino acid's carbon skeleton depends on the physiological nutritional state of the body.

Energy Generation The complete oxidation of amino acids generates energy, $\text{CO}_2/\text{HCO}_3^-$, and ammonia/ammonium. Amino acids are used for energy in the body when diets are inadequate in energy (measured in kilocalories and abbreviated kcal).

Glucose and Ketone Body Production The production of glucose from a noncarbohydrate source such as amino acids is known as gluconeogenesis. Gluconeogenesis occurs primarily in the liver but also in the kidney. The carbon skeletons of several amino acids can be used to synthesize glucose. Oxaloacetate (the carbon skeleton of aspartate) and pyruvate (the carbon skeleton of alanine) may be used to produce glucose in body cells through the process of gluconeogenesis, also discussed in Chapter 3. In addition, the carbon skeleton of asparagine can be converted into oxaloacetate, and the carbon skeletons of glycine, serine, cysteine, tryptophan, and threonine can be converted into pyruvate for glucose production in the liver.

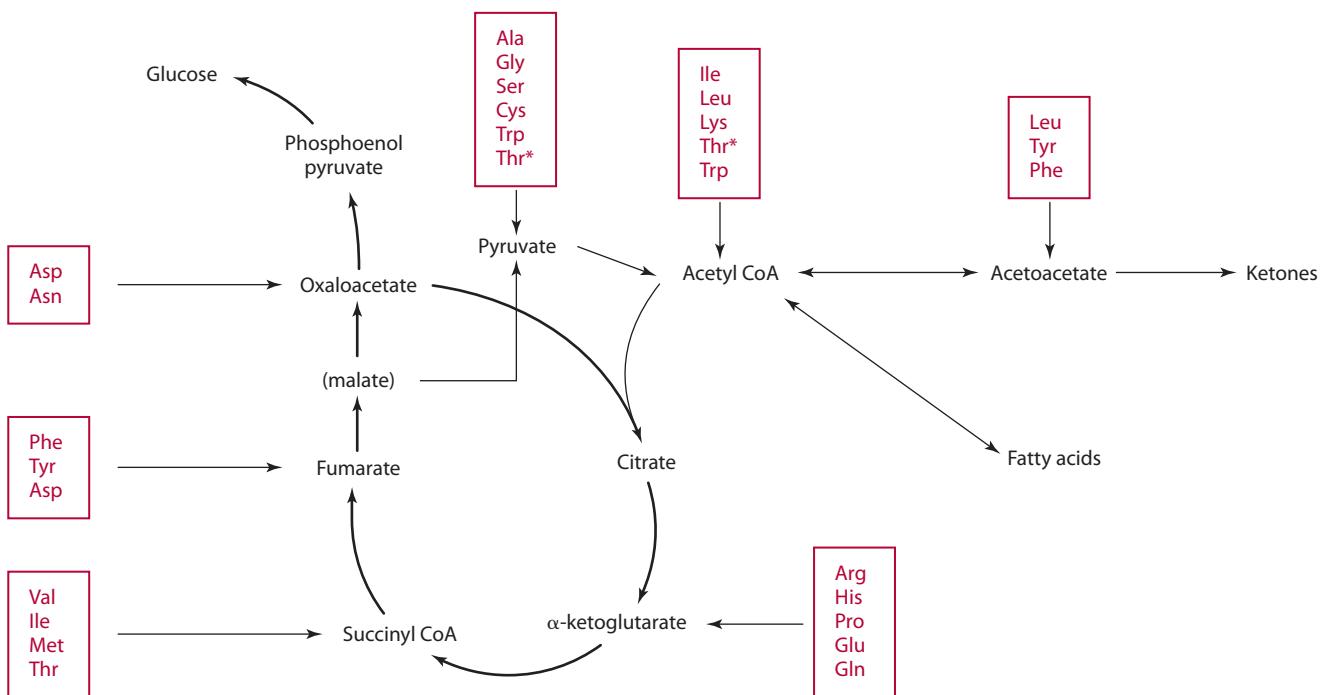
Figure 6.27 shows the general fates of amino acid carbon skeletons with respect to key intermediates of metabolism. Some amino acids, such as phenylalanine and tyrosine, can be degraded to form fumarate (an intermediate of the TCA cycle), which can be used to form glucose, but also acetoacetate, which can be used to synthesize ketone bodies. Thus, these two amino acids are both glucogenic and ketogenic. Valine and methionine are considered glucogenic, yielding succinyl CoA. Isoleucine is partially glucogenic, also generating succinyl CoA, but also ketogenic, yielding acetyl CoA as well upon its catabolism. Threonine is partially glucogenic, yielding succinyl CoA or pyruvate depending on its pathway of degradation, and partially ketogenic when degraded by another pathway to acetyl CoA. Thus, isoleucine and threonine, like phenylalanine and tyrosine, are considered partially ketogenic. Tryptophan is also considered partially ketogenic and

partially glucogenic. Tryptophan yields acetyl CoA as well as pyruvate upon catabolism.

Thus, to be considered a glucogenic amino acid, catabolism of the amino acid must yield selected intermediates of the TCA cycle. The conversion of amino acids to glucose is accelerated by high glucagon:insulin ratios and by glucocorticoids such as cortisol. Such hormones are elevated when people are not receiving sufficient energy or carbohydrate in the diet, in times of illness such as occurs with infection or trauma, or in certain disease states such as untreated diabetes mellitus and liver disease, to name a few.

For an amino acid to be considered ketogenic, the catabolism of the amino acid must generate the non-TCA cycle intermediates acetyl CoA or acetoacetate, which are used for the formation of ketone bodies. Amino acids are catabolized to generate ketone bodies generally during times when an individual is not consuming an adequate carbohydrate intake. Leucine and lysine are the only totally ketogenic amino acids and upon catabolism generate acetyl CoA.

Cholesterol Production Leucine is also the only amino acid whose catabolism generates β -hydroxy β -methylglutaryl (HMG) CoA, an intermediate in the synthesis of cholesterol. Leucine can form HMG CoA via β -hydroxy β -methylbutyrate (HMB) or β -methylglutaconyl CoA (shown later in Figure 6.37). Other amino acids, however, yield acetyl CoA, which can be metabolized in the liver to cholesterol. In muscle, leucine is thought to be an important source of cholesterol [52]. Moreover, with sufficient



*Physiological contribution unclear

Figure 6.27 The fate of amino acid carbon skeletons. Ketogenic: Lys and Leu; partially ketogenic and glucogenic: Phe, Ile, Thr, Trp, Tyr; glucogenic: Ala, Gly, Cys, Ser, Asp, Asn, Glu, Gln, Arg, Met, Val, His, Pro.

availability of HMB, it is thought that maximal cholesterol synthesis in muscles and diminishment of muscle damage and protein breakdown (especially those with exercise-induced damage) can be achieved to enable better cell growth and function [52–57].

Fatty Acid Production In times of excess energy and protein intakes coupled with adequate carbohydrate intake, the carbon skeleton of amino acids may be used to synthesize fatty acids. Leucine, for example, is used to synthesize fatty acids in adipose tissue. Its catabolism is shown later in Figure 6.37.

HEPATIC CATABOLISM AND USES OF AROMATIC AMINO ACIDS

The details of the metabolism of selected amino acids and the formation of TCA cycle and non-TCA cycle intermediates are discussed in this and the following sections. The

catabolism of the amino acids is categorized according to the structural classification of amino acids. The aromatic amino acids are discussed first followed by the sulfur-containing amino acids, the branched-chain amino acids, and then other amino acids.

The catabolism of aromatic, along with sulfur (S)-containing, amino acids occurs primarily in the liver. In fact, in end-stage (or advanced) liver disease, the inability of the liver to take up and catabolize these amino acids is evidenced by the increased plasma concentrations of both the aromatic amino acids—phenylalanine, tyrosine, and tryptophan—and the S-containing amino acids methionine and cysteine.

Phenylalanine and Tyrosine

As shown in Figure 6.28, phenylalanine and tyrosine are partially glucogenic because they are degraded to fumarate. In addition, phenylalanine and tyrosine are catabolized to acetoacetate and thus are partially ketogenic.

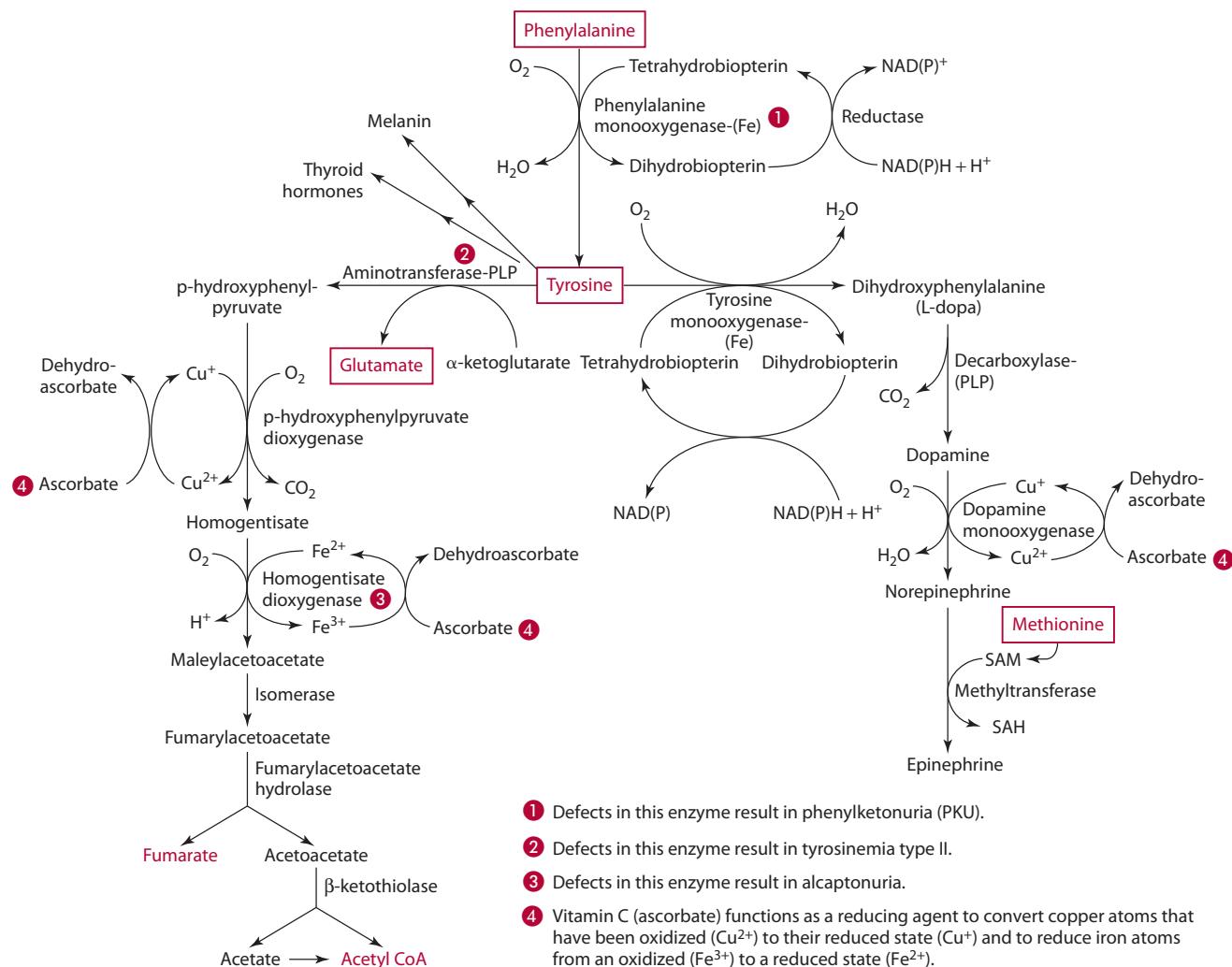


Figure 6.28 Phenylalanine and tyrosine metabolism.

- The first step in the degradation of phenylalanine is specific to the liver and the kidneys. Phenylalanine is converted to tyrosine by the enzyme phenylalanine hydroxylase, also called a monooxygenase. This enzyme is iron-dependent, and vitamin C and tetrahydrobiopterin are required for the reaction. Enzyme activity is regulated by phosphorylation/dephosphorylation with glucagon-promoting phosphorylation and enzyme activity. Insulin has the opposite effect.

Catabolism of tyrosine is not specific to the liver; however, many of the reactions for its degradation occur primarily in the liver. Other reactions, such as generation of L-dopa and the catecholamines from tyrosine, occur more commonly in neurons and in the adrenal medulla (Figure 6.28).

- Tyrosine degradation (Figure 6.28) begins with transamination by a vitamin B₆-dependent tyrosine aminotransferase to yield p-hydroxyphenylpyruvate. Higher tyrosine concentrations as well as high cortisol promote increases in tyrosine aminotransferase activity. The compound p-hydroxyphenylpyruvate, once formed, is then decarboxylated by an oxidase to generate homogentisate. Homogentisate dioxidase converts homogentisate to maleylacetoacetate, which is then isomerized to fumarylacetoacetate. A hydrolase converts fumarylacetoacetate into fumarate (a TCA cycle intermediate) and acetoacetate, which may be further metabolized to acetyl CoA.

While this section of the chapter focuses on hepatic catabolism of amino acids, it is important to remember that, tyrosine has many uses. Tyrosine is used for protein synthesis. It may be catabolized for energy, glucose, or ketone body production. Tyrosine also can be used to synthesize other compounds.

- In other cells of the body, tyrosine is used for the synthesis of L-dopa and catecholamines (Figure 6.28). The initial reaction uses tyrosine hydroxylase (also called monooxygenase), an iron-dependent enzyme. The enzyme hydroxylates tyrosine to generate 3,4-dihydroxyphenylalanine (L-dopa). Subsequent reactions utilizing L-dopa yield the catecholamines (dopamine, norepinephrine, and epinephrine).
- In melanocytes in the skin, eye, and hair cells, tyrosine is converted into melanin. The reactions occur within melanosomes, membrane-bound organelles found in the melanocytes. Melanin is a pigment that gives color to skin, eyes, and hair.
- In the thyroid gland, tyrosine is taken up and used with iodine to synthesize thyroid hormones.

Disorders of Phenylalanine and Tyrosine Metabolism Several inborn errors of metabolism have been identified in phenylalanine and tyrosine metabolism. The autosomal recessive genetic disorder phenylketonuria (PKU) occurs

when the activity of phenylalanine hydroxylase, which converts phenylalanine to tyrosine (Figure 6.28), is defective. This defect results in a buildup of phenylalanine and metabolites of phenylalanine (phenyllactate, phenylpyruvate, and phenylacetate) in the blood and other body fluids. In addition, because phenylalanine cannot be converted to tyrosine, blood concentrations of tyrosine diminish. If untreated, PKU causes neurologic problems, seizures, and hyperactivity, among other problems. The disorder is treated with a phenylalanine-restricted diet, which means that ingestion of protein-containing foods is extremely limited, and tyrosine must be added to the diet, because it cannot be made in the body. In addition, labels on products that contain aspartame (brand name Equal) must have a warning to those with PKU indicating that the product contains phenylalanine and thus must be restricted.

Impaired activity of tyrosine aminotransferase, which converts tyrosine to p-hydroxyphenylpyruvate, results in another inborn error of metabolism called tyrosinemia type II (Figure 6.28). This form of tyrosinemia is characterized by high plasma tyrosine concentrations, skin and eye lesions, and impaired mental development. People with the disorder must consume a diet restricted in both phenylalanine and tyrosine. Another genetic disorder involving tyrosine degradation is alcaptonuria, which results from defective homogentisate dioxygenase activity (Figure 6.28). This enzyme normally converts homogentisate to maleylacetoacetate. Alcaptonuria is characterized by high concentrations homogentisate in the body fluids. The homogentisate oxidizes and turns a dark color thus making the urine appear black. People with this disorder often experience joint problems as the homogentisate accumulates. Dietary treatment is not usually prescribed.

Tryptophan

Another aromatic amino acid metabolized principally by the liver is tryptophan. Its metabolism is shown in Figure 6.29. Tryptophan is partially glucogenic, because it is catabolized to form pyruvate; it is also partially ketogenic, forming acetyl CoA.

- The first step in tryptophan catabolism yields N-formylkynurenine. The enzyme tryptophan dioxygenase, which catalyzes this first reaction, is heme iron-dependent; it is also induced by glucocorticoids and glucagon. Tetrahydrobiopterin is a required cosubstrate for the reaction.
- Further catabolism of N-formylkynurenine yields formate and kynurene. Kynurene may be metabolized to 3-hydroxykynurene by a monooxygenase. The 3-hydroxykynurene may be converted to 3-hydroxyanthranilate and alanine by kynureinase, a vitamin B₆ (PLP)-dependent enzyme. Alanine formed from tryptophan degradation can be transaminated to form pyruvate, hence the glucogenic nature of tryptophan.

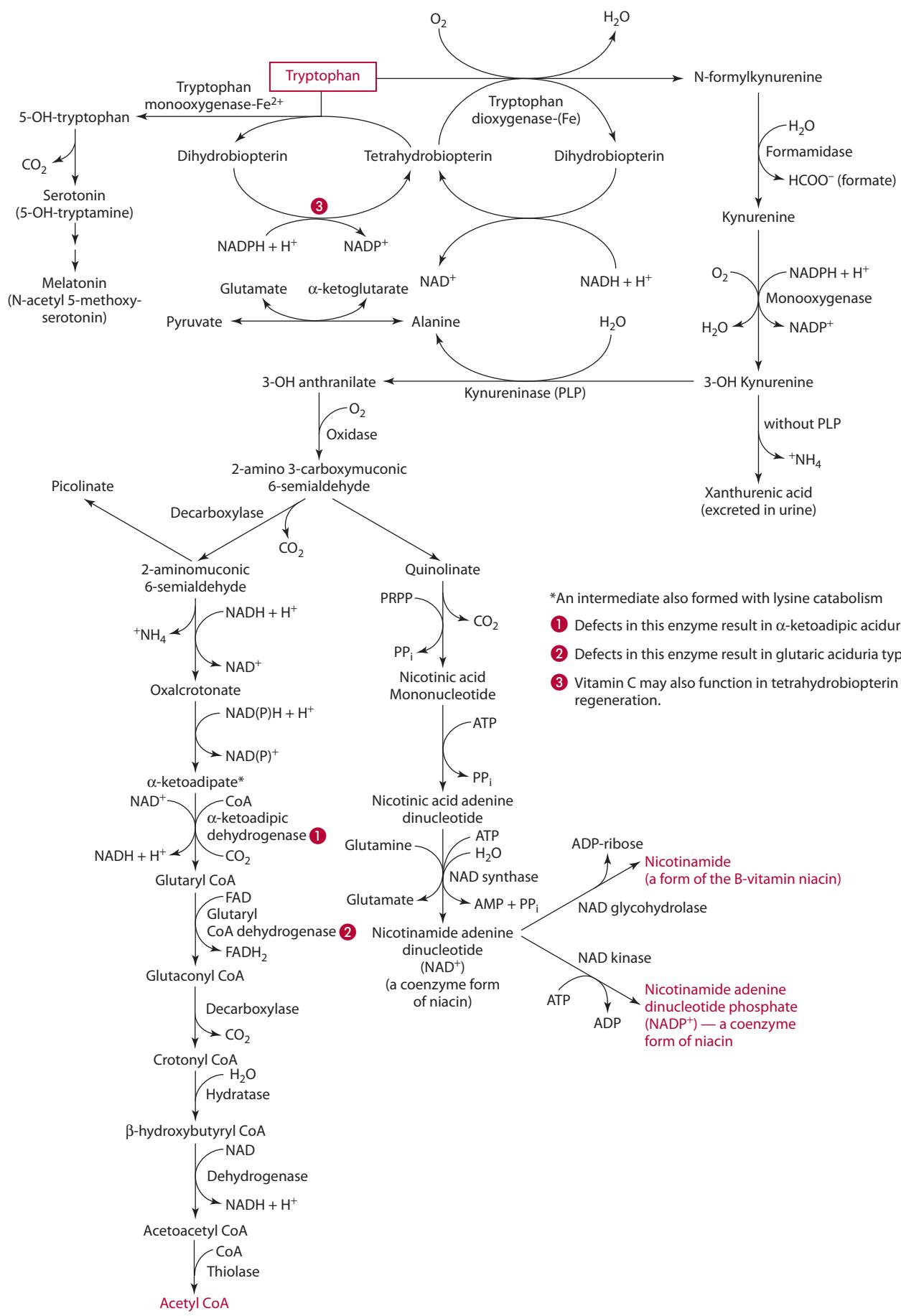


Figure 6.29 Tryptophan metabolism.

Further catabolism of 3-hydroxyanthranilate results in the formation of 2-amino 3-carboxymuconic 6-semialdehyde. This compound is further metabolized to produce many additional compounds, including picolinic acid (a possible binding ligand for minerals), niacin as nicotinamide as well as its coenzyme form nicotinamide adenine dinucleotide (NAD) phosphate (NADP), and 2-aminomuconic 6-semialdehyde, which is further metabolized by several reactions to acetyl CoA.

- In addition to use for protein synthesis and for energy, glucose, and ketone body production, tryptophan is used for the synthesis of serotonin (5-hydroxytryptamine) and melatonin (N-acetyl 5-methoxyserotonin), described later in this chapter under “Brain and Accessory Tissues.”

Disorders of Tryptophan Metabolism As part of tryptophan’s degradative pathway in which 2-aminomuconic 6-semialdehyde is converted in multiple reactions to acetyl CoA, defective activities of α -ketoadipic dehydrogenase and glutaryl CoA dehydrogenase have been demonstrated. One genetic disorder, α -ketoadipic aciduria, results from the defective activity of α -ketoadipic dehydrogenase, which converts α -ketoadipate to glutaryl CoA. With this disorder, lysine, tryptophan, α -amino adipate, α -ketoadipate, and α -hydroxyadipate build up in the blood and other body fluids. Infants with this disorder become hypotonic, acidotic, and experience seizures and a number of motor and developmental delays. Nutrition support requires a lysine- and tryptophan-restricted diet, because this set of reactions is common in both tryptophan and lysine degradation. A second disorder in this pathway also occurs. Glutaric aciduria type 1, an autosomal recessive condition, results from the defective activity of the riboflavin-dependent enzyme glutaryl CoA dehydrogenase, which converts glutaryl CoA to glutaconyl CoA. As with α -ketoadipic aciduria, the enzyme glutaryl CoA dehydrogenase is critical to the catabolism of tryptophan (Figure 6.29) as well as lysine (Figure 6.31). In glutaric aciduria type 1, glutaryl CoA builds up and is converted to glutaric acid, which also accumulates in body fluids. Over time, affected infants develop acidosis, ataxia, seizures, and macrocephaly, among other problems. Treatment requires a diet restricted in both lysine and tryptophan (because both produce glutaryl CoA). In some cases riboflavin supplements can be beneficial, because the enzyme is riboflavin dependent.

HEPATIC CATABOLISM AND USES OF SULFUR (S)-CONTAINING AMINO ACIDS

The catabolism of methionine, an S-containing essential amino acid, occurs to a large extent in the liver and generates the S-containing nonessential amino acids cysteine and taurine and the methyl donor S-adenosyl methionine (SAM). Methionine metabolism is shown in Figure 6.30 and briefly described here.

Methionine

- The first step in methionine catabolism (required for the use of methionine’s methyl group) is the conversion of methionine to S-adenosyl methionine (SAM) by methionine adenosyl transferase (present in high concentrations in the liver) in an ATP-requiring reaction. SAM has many functions in the body. SAM promotes methionine metabolism; it stimulates cystathione synthase, which converts homocysteine to cystathione. SAM also inhibits methylene THF reductase activity, which forms N5-methyl THF needed to regenerate methionine from homocysteine. Thus, SAM (when present in higher concentrations) facilitates the degradation of methionine and not its resynthesis. SAM also has other functions. SAM serves as the principal methyl donor in the body and, as such, is required for the synthesis of carnitine, creatine, epinephrine, purines, sarcosine, and nicotinamide. Furthermore, the methyl groups from SAM are used to methylate DNA, and thus affect gene expression. In addition, SAM may be decarboxylated to form S-adenosyl methylthiopropylamine, an intermediate in the synthesis of the polyamines—putrescine, spermidine, and spermine. Polyamines are important in cell division and growth. The removal or donation of the methyl group from SAM yields the compound S-adenosyl homocysteine (SAH).
- SAH can be converted to homocysteine by the enzyme S-adenosyl homocysteine hydrolase. Homocysteine can be converted back to methionine in a betaine-dependent reaction or a vitamin B₁₂ (as methylcobalamin) and folate (as 5-methyl tetrahydrofolate)–dependent reaction. Betaine, generated in the liver from choline, provides a methyl group that is transferred to homocysteine by the hepatic enzyme betaine homocysteine methyltransferase. With the loss of the methyl group, betaine becomes dimethylglycine. Dimethylglycine can be further demethylated to generate glycine. In the vitamin B₁₂ and folate–dependent remethylation reaction (Figure 6.30), methylcobalamin directly provides the methyl group to remethylate homocysteine to form methionine. Methylcobalamin receives the methyl group from 5-methyl tetrahydrofolate (a coenzyme form of the B vitamin folate). Elevated levels of homocysteine in the blood have been associated with interference with collagen cross-linking in bone and may increase fracture risk. High plasma homocysteine concentrations also have been found to be a risk factor for heart disease and may develop if folate, vitamin B₁₂, or vitamin B₆ status is poor. A discussion of the importance of adequate folate, vitamin B₁₂, and vitamin B₆ nutriture and heart disease is found in Chapter 9 in the sections on folic acid and vitamin B₁₂.
- To be further metabolized in the body, homocysteine must react with the amino acid serine, forming cystathione through the action of cystathione synthase.

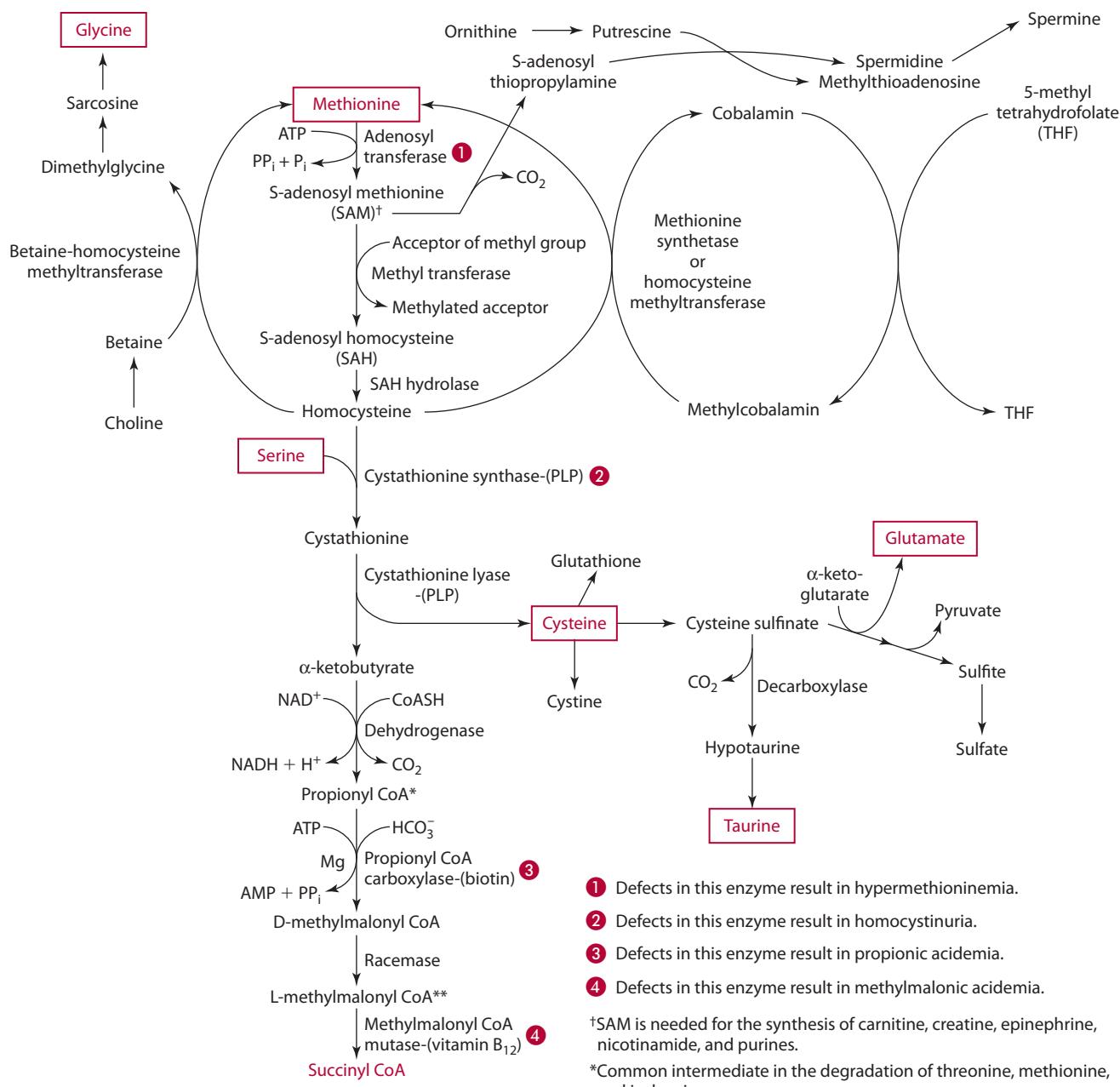


Figure 6.30 Methionine and cysteine metabolism.

The presence of vitamin B₆ in its coenzyme form (PLP) is necessary for this reaction to occur, hence the need for adequate vitamin B₆ status to prevent elevated blood homocysteine concentrations.

■ Further catabolism of cystathione requires additional coenzymes. Cystathione is cleaved by cystathione lyase, another vitamin B₆-dependent enzyme, to form the dispensable amino acid cysteine. Also generated in the reaction is α-ketobutyrate, which is further decarboxylated to propionyl CoA. The conversion of homocysteine to cysteine by cystathione synthase and cystathione lyase is sometimes called the

transulfuration pathway. These reactions occur in the liver, but also in the kidney, intestine, and pancreas.

■ Propionyl CoA (made from α-ketobutyrate) is next converted to D-methylmalonyl CoA by the biotin-dependent enzyme propionyl CoA carboxylase. D-methylmalonyl CoA is then converted to L-methylmalonyl CoA by a racemase. Then L-methylmalonyl CoA is converted by methylmalonyl CoA mutase, a vitamin B₁₂-dependent enzyme, to the TCA cycle intermediate succinyl CoA.

Disorders of Methionine Metabolism Defects in methionine adenosyl transferase, the enzyme converting methionine to

S-adenosyl methionine (SAM), result in genetic disorder hypermethioninemia. This condition is characterized by high blood concentrations of methionine and thus treatment necessitates a diet restricted in methionine but containing increased cysteine.

Defects in cystathione synthase result in the genetic disorder homocystinuria. Cystathione synthase converts homocysteine to cystathione. People with this condition exhibit high blood homocysteine and methionine concentrations and low cysteine concentrations. The high homocysteine concentrations promote over time the formation of blood clots (thrombi) and subsequent organ damage. Other manifestations include skeletal problems, osteoporosis, ocular changes, and mental retardation among other problems. Treatment requires a diet low in methionine (and thus low intakes of normal protein-containing foods), added cysteine, and in some cases supplements of betaine and folate.

The genetic disorder propionic acidemia results from defects in the activity of propionyl CoA carboxylase, a biotin-dependent enzyme. Another disorder caused by genetic errors in the same pathway, methylmalonic acidemia results from impaired methylmalonyl CoA mutase activity. Propionic acidemia is characterized by the accumulation of propionic acid in body fluids, and in methylmalonic acidemia both propionic and methylmalonic acids accumulate in body fluids (as well as other compounds such as methylcitrate, 3-hydroxy propionate, and tiglic acid). Infants exhibit excessive vomiting, ketoacidosis, hypertonia, failure to thrive, and respiratory difficulties, among other problems. Because propionyl CoA and methylmalonyl CoA are generated from not only methionine as shown in Figure 6.30, but also from the degradation of threonine (shown later in Figure 6.32), isoleucine, and valine (see later Figure 6.38), the diets of people with either of these conditions require restriction of all four amino acids. In addition, odd-chain fatty acids and polyunsaturated fatty acids (in excessive amounts) also can generate propionyl CoA and thus must be restricted. In some cases, biotin supplements have been shown to improve the activity of propionyl CoA carboxylase, but the people usually still require restricted diets. Similarly, vitamin B₁₂ supplements can sometimes improve methylmalonyl CoA mutase activity in some people with methylmalonic acidemia (remember methylmalonyl CoA mutase is vitamin B₁₂-dependent).

Cysteine

- Cysteine is a nonessential amino acid. Hepatic concentrations of free cysteine appear to be tightly controlled. Cysteine is used like other amino acids for protein synthesis. It is also used to synthesize glutathione, whereby glutamate cysteine ligase attaches cysteine and glutamate in the first step of glutathione synthesis. Cysteine is also converted by cysteine dioxygenase to cysteine

sulfinate, which is used to produce the amino acid taurine (Figure 6.30).

- Taurine, a β-amino sulfonic acid, is made in the liver but concentrated in muscle and the central nervous system; it is also found in smaller amounts in the heart, liver, and kidney, among other tissues. Although taurine is not involved in protein synthesis, it is important in the retina, where it is thought to exhibit antioxidant properties and help maintain the structure and function of the photoreceptor cells. Taurine is thought to maintain membrane stability by scavenging peroxidative (e.g., oxychloride) products. Taurine also functions in the liver and intestine as a bile salt, taurocholate, and in the central nervous system as an inhibitory neurotransmitter.
- Cysteine degradation (Figure 6.30) yields pyruvate and sulfite. Sulfite is converted by sulfite oxidase (an iron- and molybdenum-dependent enzyme) to sulfate, which can be excreted in the urine or used in to synthesize sulfolipids and sulfoproteins.

HEPATIC CATABOLISM AND USES OF THE BRANCHED-CHAIN AMINO ACIDS

The liver plays only a minor role in the initial catabolism of the three branched-chain amino acids, isoleucine, leucine, and valine. Transaminase activity needed to remove the amino groups is minimal in the liver, although hepatic transferases increase in response to glucocorticoid (cortisol) release, which occurs in situations such as stress, trauma, burns, and sepsis. Thus, under normal circumstances, the branched-chain amino acids typically remain in circulation and are taken up and transaminated primarily by the skeletal muscle, but also by the heart, kidney, diaphragm, and adipose, if needed. Alpha keto-acids of the branched-chain amino acids, generated from branched-chain amino acid transamination, may be used within the tissues or released into circulation. The liver, among other organs, can further catabolize these α keto-acids. Additional information on branched-chain amino acid metabolism is found under “Skeletal Muscle” in the section of this chapter titled “Interorgan ‘Flow’ of Amino Acids and Organ-Specific Metabolism.”

HEPATIC CATABOLISM AND USES OF OTHER AMINO ACIDS

Several other reactions of amino acid catabolism are confined primarily to the liver. Some of the pathways are presented in this section.

Lysine

The catabolism of lysine, a totally ketogenic amino acid, generates acetyl CoA as shown in Figure 6.31. Lysine and tryptophan degradation share a common intermediate,

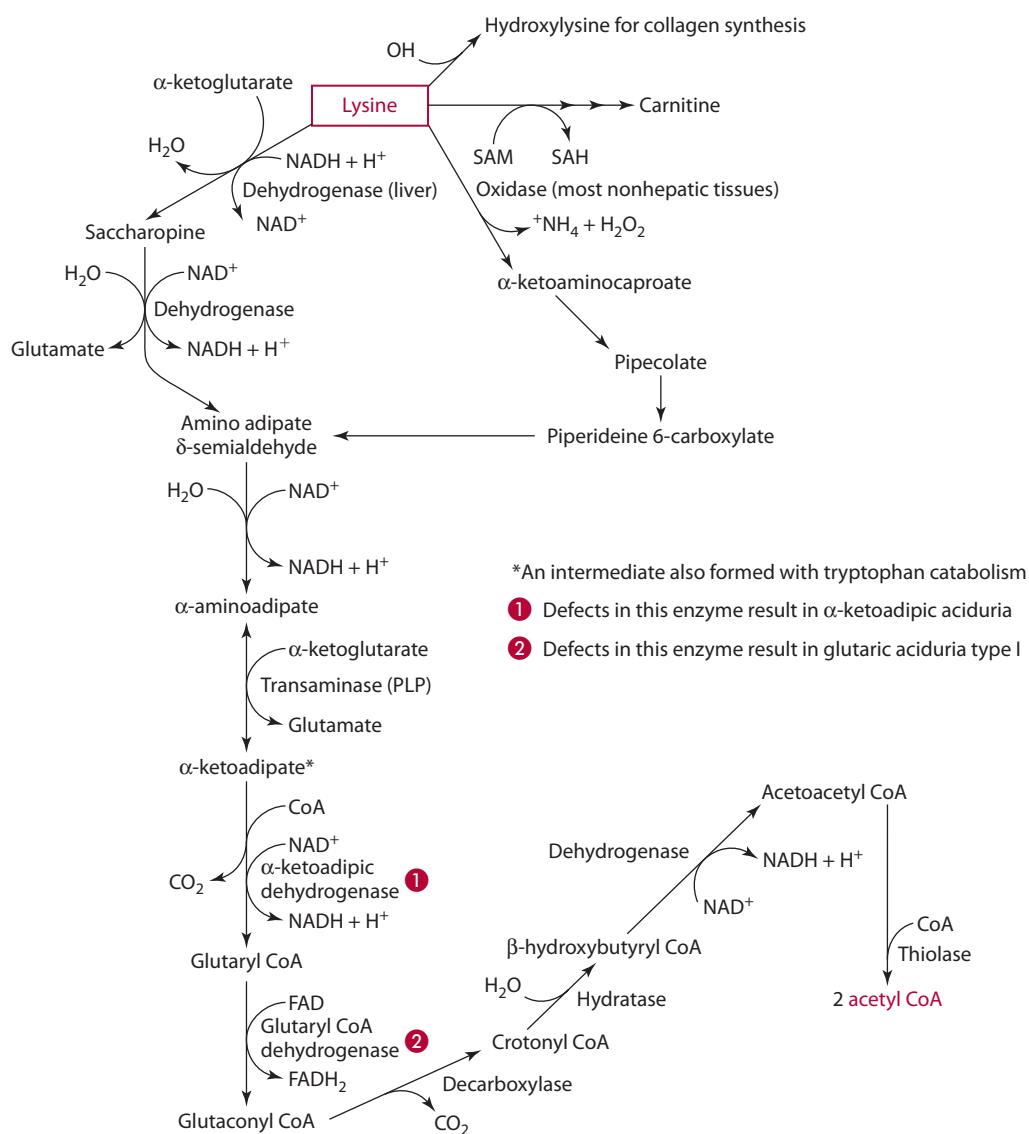


Figure 6.31 Lysine metabolism.

α-ketoacidipate, and thus share some common reactions. Note that lysine (after being methylated using SAM) is used in the synthesis of carnitine, which is needed for fatty acid oxidation.

Disorders of Lysine Metabolism Defects in lysine degradation by glutaryl CoA dehydrogenase and α-ketoacidipate dehydrogenase result in glutaric aciduria type 1 and α-ketoacidipic aciduria, respectively, as discussed under “Disorders of Tryptophan Metabolism.”

Threonine

Threonine can be metabolized by three different pathways. One of the more commonly used pathways of degradation is through cytosolic threonine dehydratase to generate α-ketobutyrate, which is further catabolized to propionyl CoA, then to D-methylmalonyl CoA, L-methylmalonyl CoA, and ultimately succinyl CoA as shown in Figure 6.32.

These latter steps in the catabolism are shared with methionine, isoleucine, and valine. Alternately, threonine may be degraded by mitochondrial threonine dehydrogenase to form aminoacetone, which is converted to methylglyoxal and then pyruvate. This pathway is thought to be used if threonine concentrations are relatively high. In a third pathway, the mitochondrial threonine cleavage complex (composed of a dehydrogenase and a ligase) converts threonine to glycine and acetylaldehyde; acetylaldehyde is further metabolized to acetate and then to acetyl CoA in an ATP- and CoA-dependent reaction (Figure 6.32). Threonine is used to synthesize body proteins and nitrogen-containing nonproteins and is found in fairly high concentrations relative to other amino acids in mucus glycoproteins.

Disorders of Threonine Metabolism Defects in two steps of threonine metabolism from propionyl CoA to succinyl CoA can lead to propionic acidemia and methylmalonic

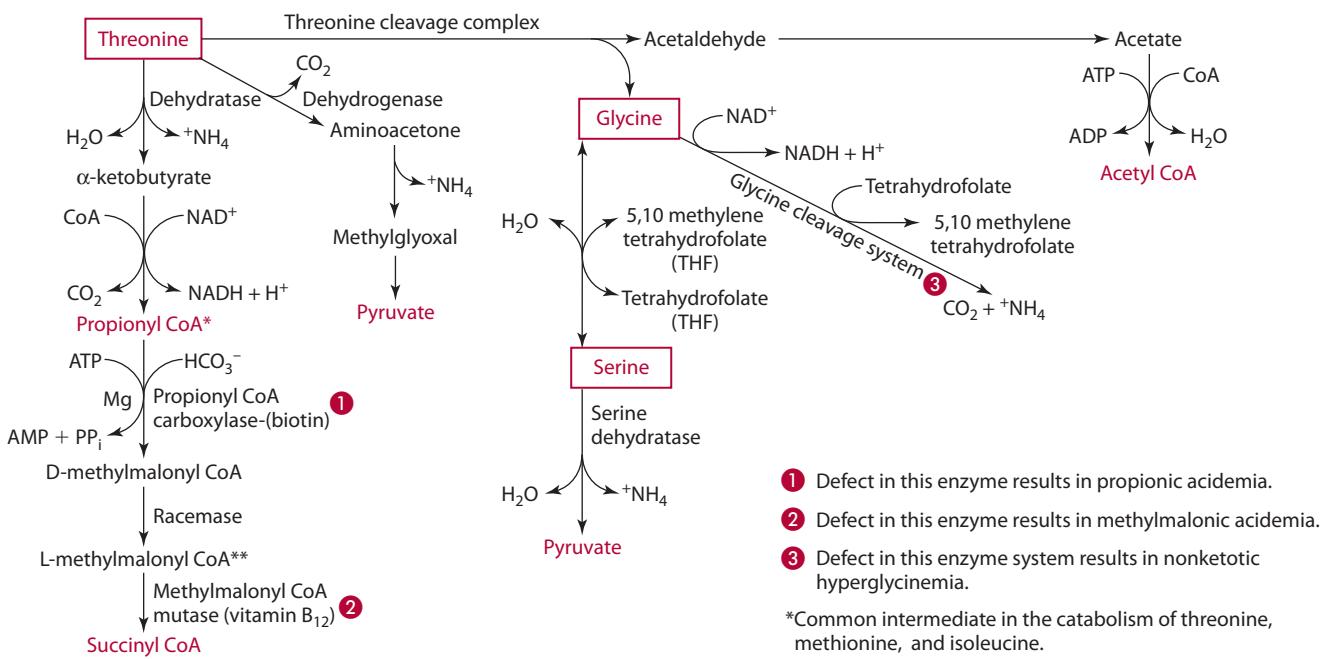


Figure 6.32 Threonine, glycine, and serine metabolism.

acidemia. See the section on “Disorders of Methionine Metabolism.”

Glycine and Serine

Glycine and serine are produced from one another in a reversible reaction that requires folate. Glycine is converted to serine mainly in the kidney (Figure 6.32). Glycine, however, is also needed for the synthesis of other important body compounds, including creatine, heme/porphyrins (nitrogen and iron-containing nonprotein portions of hemoglobin), sarcosine, and the bile salt glycocholate. Serine is used for the synthesis of ethanolamine and choline for phospholipids. See the section on the kidney under “Interorgan ‘Flow’ of Amino Acids and Organ-Specific Metabolism.”

Disorders of Glycine Metabolism Impaired catabolism of glycine, caused by an autosomal recessive defect in the mitochondrial glycine cleavage system, results in nonketotic hyperglycinemia. This enzyme complex normally converts glycine into ammonia and carbon dioxide and requires folate as tetrahydrofolate (Figure 6.32). Defects in four different genes that code for the complex have been reported. Infants with the condition exhibit seizures, neurologic deterioration, flaccidity, and lethargy, among other problems. Blood and other body fluids contain increased glycine concentrations. A low-protein diet is necessary for people with nonketotic hyperglycinemia.

Arginine

Arginine is metabolized mostly in the liver and kidney, but also in the intestine. In the kidney, arginine is used with glycine in the first reaction of creatine synthesis. It

is also released for use by body cells. (See the section on the kidney under “Interorgan ‘Flow’ of Amino Acids and Organ-Specific Metabolism.”) In the liver, arginine catabolism is used to generate urea, as part of the urea cycle, and ornithine. Ornithine may be decarboxylated to form the polyamine putrescine or may be transaminated by ornithine aminotransferase to form glutamate γ-semialdehyde, which may be converted to glutamate or to pyrrolidine 5-carboxylate. Pyrrolidine 5-carboxylate may then be metabolized to form proline (Figure 6.33), which also may be converted to glutamate. In addition, arginine is used for nitric oxide production in endothelial cells, cerebellar neurons, neutrophils, and splanchnic tissues. Nitric oxide is involved in the regulation of a variety of physiological processes including regulation of blood pressure (relaxation of vascular smooth muscle) and intestinal motility, inhibition of platelet aggregation, and macrophage function. Nitric oxide also can combine with glutathione to form glutathionylated nitric oxide, a compound that in turn is known to stimulate protein glutathionylation. This posttranslational modification is thought to affect the function and stability of many cellular proteins.

Histidine

Histidine degradation is also shown in Figure 6.33. Histidine may be catabolized to form glutamate or histidine may combine with β-alanine to generate carnosine (a nitrogen-containing nonprotein compound). Through a vitamin B₆-dependent decarboxylation reaction, the amine histamine also can be formed from histidine (Figure 6.33). Histamine is found in neurons, in cells of the gastric mucosa, and in mast cells. Histamine release

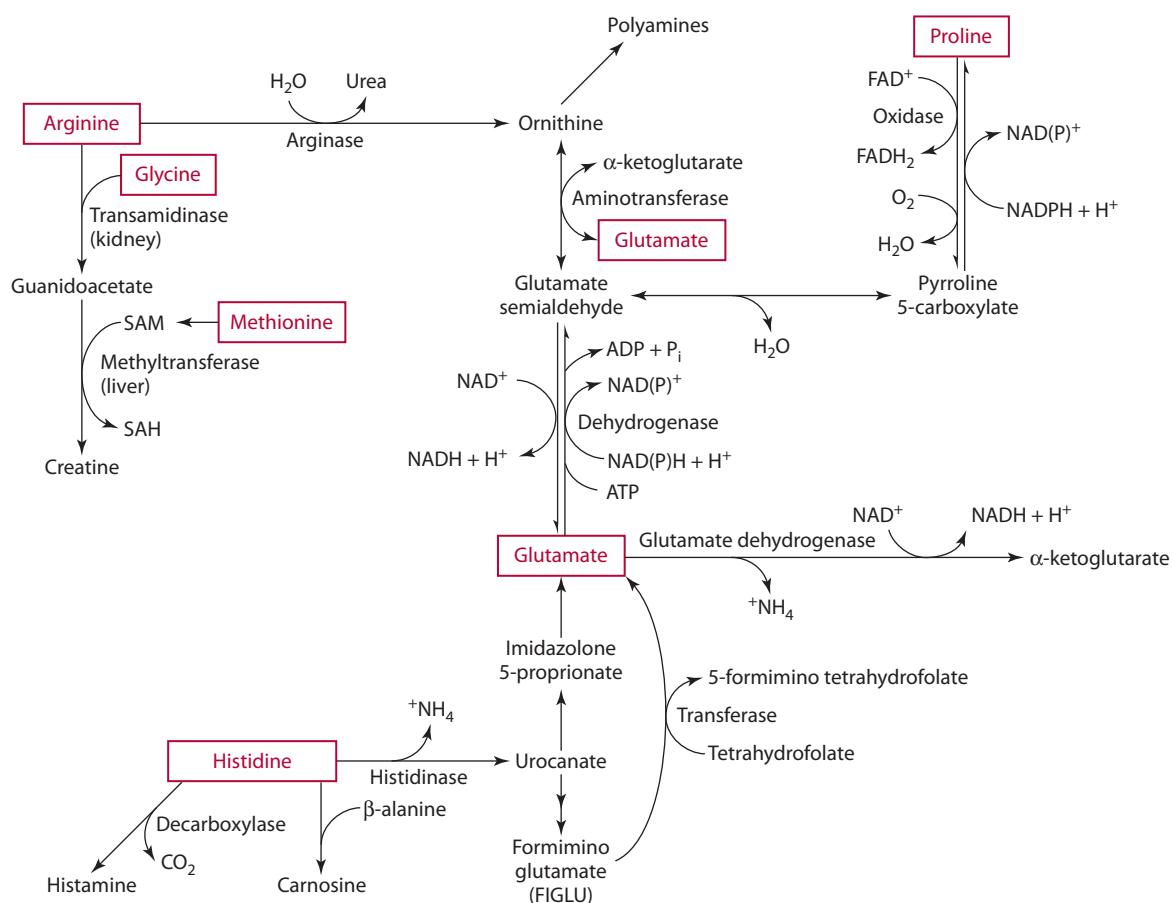


Figure 6.33 Arginine, proline, histidine, and glutamate metabolism.

causes dilation of capillaries (flushing of the skin), constriction of bronchial smooth muscle, and increased gastric secretions. Figure 6.34 provides an overview of the fates of amino acids not used for the synthesis of body proteins.

AMINO ACIDS NOT TAKEN UP BY THE LIVER: PLASMA AMINO ACIDS AND AMINO ACID POOL(S)

Ingestion of protein-containing meals is followed by a rise in plasma amino acid concentrations, especially concentrations of the branched-chain amino acids, which are released from the liver without being metabolized. After a meal, amino acid concentrations typically rise in the plasma for several hours, then return to basal concentrations. In basal situations or between meals, plasma amino acid concentrations are relatively stable and are species-specific; however, absolute concentrations of specific amino acids in the plasma vary substantially from person to person.

Amino acids circulating in the plasma and found within cells arise from digestion and absorption of dietary protein as well as from the breakdown of existing body tissues. These endogenous amino acids intermingle with exogenous amino acids to form a “pool” totaling about 150 g. The pool includes amino acids in the plasma as well as amino acids in various tissues of the body. Reuse of endogenous amino acids is thought to represent the primary source of amino acids needed for protein synthesis. Despite differences in protein intake and rate of degradation of tissue protein, the pattern of the amino acids in the free amino acid pool appears to remain relatively constant, although the pattern is quite different from that found in body proteins.

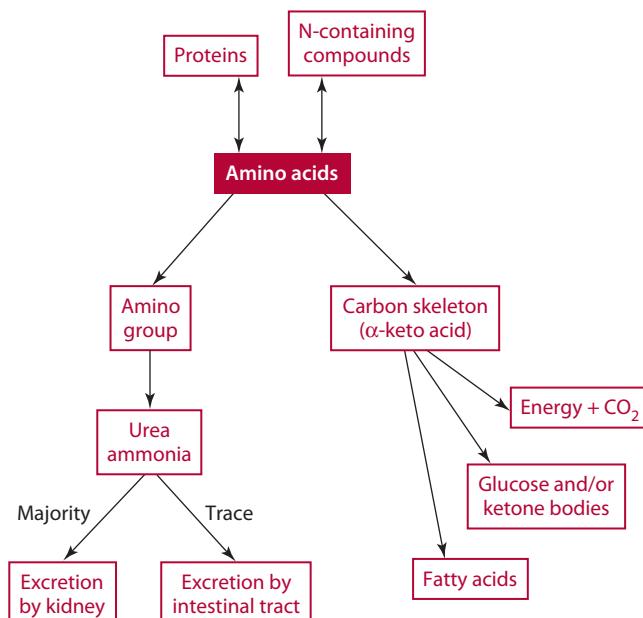


Figure 6.34 Possible fates of amino acids upon catabolism.

The total amount of the essential amino acids found in the pool is less than that of the nonessential amino acids. The essential amino acids found in greatest concentration are lysine and threonine, both of which are totally indispensable. Of the nonessential amino acids, those found in greatest concentration are alanine, glutamate, aspartate, and glutamine. The amino acid found in greatest quantities (up to 80 g) in the pool is glutamine. The nonessential amino acids may function to conserve the essential amino acids, with the exception of histidine, lysine, and threonine, through the reamination of α -keto acids of the essential amino acid, as discussed earlier in this chapter.

Amino acids within the pool, regardless of source, are metabolized in response to various stimuli such as hormones and physiological state. Amino acids in excess of the amount needed to synthesize protein and nonprotein nitrogen-containing compounds are likely to be oxidized. Remember, if energy intake is inadequate, amino acids can be degraded to generate energy (ATP). Glucose and ketone bodies are produced from amino acids when carbohydrate intake is insufficient. Cholesterol synthesis from some amino acids can also occur, and fatty acids can be made from amino acids in some tissues when energy intake is in excess of the body's energy needs. The ratio of glucagon to insulin, as well as other hormones, is an important determinant of the fate of the amino acids.

Interorgan “Flow” of Amino Acids and Organ-Specific Metabolism

From the plasma, tissues extract amino acids for energy production or for the synthesis of nonessential amino acids, protein, nonprotein nitrogen-containing compounds, biogenic amines and neurotransmitters, hormones and peptides, glucose, fatty acids, or ketones, depending on the person's nutritional status and hormonal environment. The next section briefly reviews the flow of amino acids between selected organs and organ-specific amino acid metabolism.

GLUTAMINE AND THE LIVER, KIDNEYS, AND INTESTINE

Glutamine provides several major functions in the body. One of these functions is ammonia transport. Whereas ammonia arising in the liver from amino acid reactions can be readily shuttled into the urea cycle, this is not true in other tissues. In extrahepatic tissues, ammonia (NH_3) or ammonium ions (NH_4^+) generated in the cell from amino acid reactions generally combine with the amino acid glutamate to form glutamine (Figure 6.35). This reaction

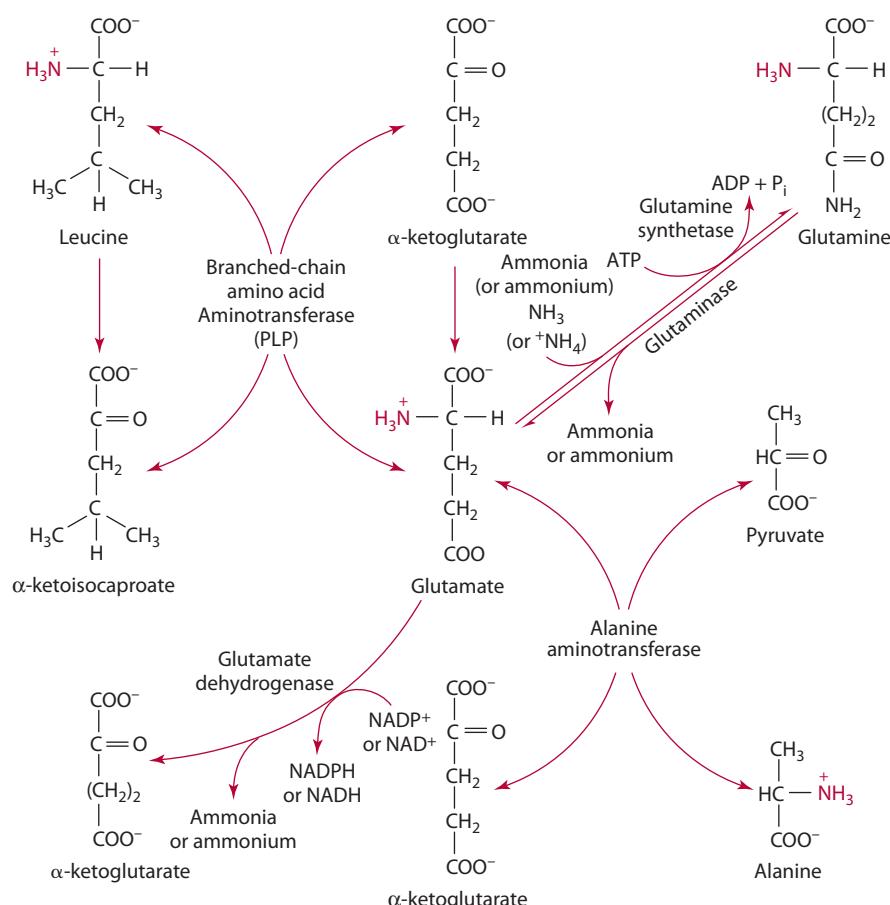


Figure 6.35 Alanine, glutamate, and glutamine generation in body cells.

is catalyzed by glutamine synthetase and requires ATP and magnesium (Mg^{2+}) or manganese (Mn^{2+}). Glutamine synthesis occurs within all tissues, including the brain and adipose, but especially large amounts are produced by the muscle and lungs. The ability of glutamine to carry the generated ammonia safely out of the cell is critical as too much free ammonia is toxic to cells!

Glutamine freely leaves tissues, and it travels principally to the liver, kidney, and intestine but also to organs such as the pancreas. Whereas the cells of the gastrointestinal tract, as well as the immune system (lymphocytes and macrophages) rely on glutamine catabolism for energy production, glutamine in the liver and kidney is catabolized by glutaminase, which removes the amide nitrogen to yield glutamate and ammonia (Figure 6.35). The fate of the ammonia varies. In the absorptive state (or during periods of alkalosis), liver glutaminase activity increases, yielding ammonia for the urea cycle. In the urea cycle, ammonia reacts with HCO_3^-/CO_2 to form carbamoyl phosphate, which is made approximately in proportion to the ammonia concentration. If the periportal hepatocytes fail to capture the ammonia for ureagenesis, the ammonia is quickly taken up by perivenous hepatocytes for glutamine synthesis. In an acidotic state, the use of glutamine for the urea cycle diminishes, and the liver releases glutamine into the blood for transport to and uptake by the kidney. Glutamine is catabolized by renal tubular glutaminase to yield ammonia and glutamate. Glutamate may be further catabolized by glutamate dehydrogenase to yield α -ketoglutarate plus another ammonia (Figure 6.35). In the kidney, ammonium concentrations are in equilibrium with cell ammonia and H^+ . Ammonia, which is lipid soluble, may diffuse into the urine and react with H^+ to form ammonium for excretion (shown later in Figure 6.40). Renal glutaminase activity and ammonium excretion increase with acidosis and decrease with alkalosis. Thus, glutamine, by virtue of its ubiquitous synthesis in cells and its ability to diffuse out of cells for transport to tissues, is a major carrier of nitrogen between cells.

Glutamine use increases dramatically during hypercatabolic conditions such as sepsis and trauma. In these conditions muscle glutamine release increases but cannot meet cellular demands. Thus, glutamine stores become depleted and cell functions become compromised [16]. Remember, glutamine also promotes the synthesis of heat shock or stress proteins, promotes white blood cell proliferation, serves as a substrate for energy production for cells of the intestine and immune system, and, along with alanine, upon uptake into cells promotes increases in cell volume with possible associated regulatory roles in intermediary metabolism.

ALANINE AND THE LIVER AND MUSCLE

In addition to glutamine, another amino acid, alanine, also is important in the intertissue (between tissue) transfer of amino groups generated from amino acid catabolism. For example, amino groups generated from branched-chain

amino acid transamination in tissues such as the skeletal muscle can combine with α -ketoglutarate to form glutamate. In Figures 6.35 and 6.36, leucine is transaminated with α -ketoglutarate by branched-chain amino acid transaminase to form the α -keto acid α -ketoisocaproate and the amino acid glutamate. Glutamate may accept another amino group to form glutamine or transfer its amino group to pyruvate, generated from glucose metabolism, to form alanine (Figure 6.35). Extrahepatic tissues, such as muscle, often release glutamine and alanine into the blood. Alanine that is released from muscle usually travels to the liver (Figure 6.36). Within the liver, alanine may undergo transamination. Alanine transamination with α -ketoglutarate produces glutamate. Glutamate may be deaminated to yield ammonia for the urea cycle, or it may be transaminated with oxaloacetate to form aspartate (Figure 6.25). Aspartate is used in the synthesis of pyrimidines and purines and is one of the amino acids directly involved in urea generation by the urea cycle.

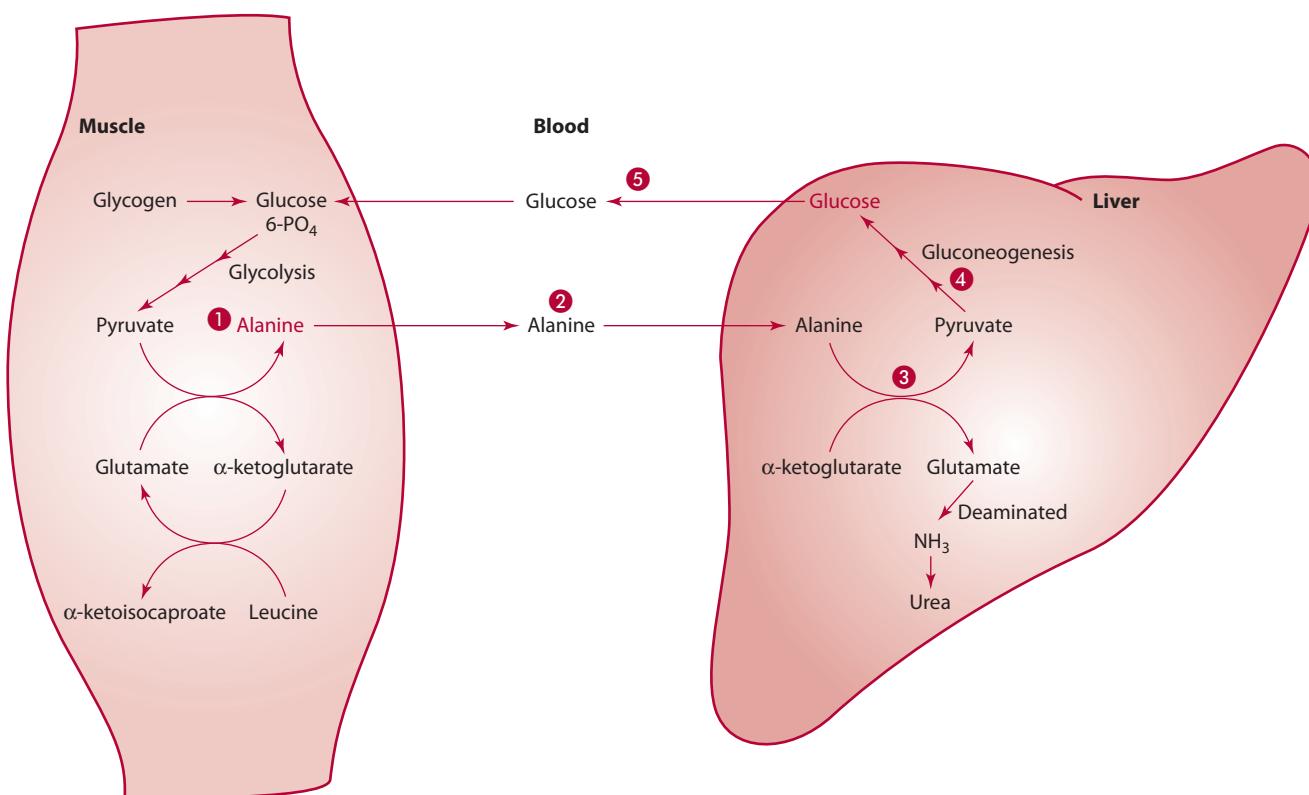
Alternately, alanine within the liver can be converted to glucose. These reactions (Figure 6.36), known as the glucose-alanine cycle or the alanine-glucose cycle, occur especially in situations marked by low carbohydrate stores (low liver glycogen) to maintain blood glucose or by excessive use or need for glucose. Typically, the cycle is more active when blood glucagon and possibly epinephrine and cortisol concentrations are elevated. The glucose that is generated from the alanine is subsequently released into the blood, where it is available to be taken up again and used by muscle. Muscle cells use the glucose through glycolysis and generate pyruvate. The formed pyruvate is again available for transamination with glutamate. This alanine-glucose cycle serves to transport nitrogen to the liver for conversion to urea while also allowing needed substrates to be regenerated.

SKELETAL MUSCLE

About 40% of the body's protein is found in muscle, and skeletal muscle mass represents about 43% of the body's mass. Uptake of amino acids by the skeletal muscles readily occurs following ingestion of a protein-containing meal. During this time, skeletal muscles typically experience a net protein synthesis (e.g., protein synthesis is greater than protein degradation). With respect to amino acid degradation, six amino acids (aspartate, asparagine, glutamate, leucine, isoleucine, and valine) appear to be catabolized to greater extents in the skeletal muscle than other tissues. The catabolism of aspartate, asparagine, and glutamate was presented earlier in the chapter. A brief review of branched-chain amino acid (isoleucine, leucine, valine) catabolism follows and is outlined in Figure 6.37.

Isoleucine, Leucine, and Valine

Muscle, as well as the heart, kidney, diaphragm, adipose, and other organs (except, for the most part, the liver),



- ① Alanine is formed in muscle cells from transamination with glutamate (generated from leucine transamination) and from pyruvate (generated from glucose oxidation via glycolysis).
- ② Alanine travels in the blood to the liver.
- ③ In the liver, alanine is transaminated with α-ketoglutarate to form pyruvate.
- ④ Pyruvate can be converted back to glucose in a series of reactions.
- ⑤ The glucose is released from the liver into the blood for uptake by tissues such as muscle which use glucose for energy.

Figure 6.36 The alanine–glucose cycle: alanine generation in muscle, glucose generation in the liver.

possesses branched-chain aminotransferases, located in both the cytoplasm and mitochondria. These aminotransferases (transaminases) are needed for the transamination of the three branched-chain amino acids; the reactions catalyzed by the aminotransferases are reversible. Following transamination, the α-keto acids of the branched-chain amino acids may remain within muscle for further oxidation or may be transported (bound to albumin) in the blood to other tissues (including the liver) for reamination or further catabolism.

The next step following the transamination of isoleucine, leucine, and valine catabolism is the decarboxylation (an irreversible reaction) of the α-keto acids by the branched-chain α-keto acid dehydrogenase (BCKAD) complex. BCKAD is a large multienzyme complex made up of three subunits: E1α, E1β, and E2. This enzyme complex is found in the mitochondria of many tissues, including liver, muscle, heart, kidney, intestine, and brain. It is highly regulated through phosphorylation (inactivation) and dephosphorylation (activation) mechanisms involving kinase and phosphatase proteins that act on the E1α

subunit and through end product inhibition. This enzyme operates in a fashion similar to pyruvate dehydrogenase complex (see Chapter 3) in that it requires thiamin in its coenzyme form TDP/TPP, niacin as NADH, and Mg²⁺ and CoA(SH) from pantothenic acid and it is affected in some tissues by changes in dietary protein intake [58]. A genetic defect diminishing branched-chain α-keto acid dehydrogenase complex activity results in maple syrup urine disease (MSUD). MSUD necessitates a diet restricted in leucine, isoleucine, and valine intakes.

As shown in Figure 6.37, the complete oxidation of valine yields succinyl CoA. Thus, valine is considered glucogenic. The end products of isoleucine catabolism are succinyl CoA and acetyl CoA, which are glucogenic and ketogenic, respectively. Isoleucine oxidation also generates propionyl CoA, which is a common intermediate in the degradative pathways of methionine and threonine. Valine oxidation generates methylmalonyl CoA, a common intermediate in the degradative pathways of methionine, threonine, and isoleucine. Methylmalonyl CoA is converted to the TCA cycle intermediate succinyl CoA. As discussed

under “Disorders of Methionine Metabolism”, people with propionic acidemia and methylmalonic acidemia must restrict dietary intakes of valine, isoleucine, threonine, and methionine.

Complete oxidation of leucine results in acetyl CoA and acetoacetate formation, and acetoacetate may be further metabolized to form acetyl CoA (Figure 6.37). Leucine thus is totally ketogenic. Leucine’s metabolism also generates β -hydroxy β -methylbutyrate (HMB). This compound appears to be important for both cholesterol synthesis in the muscle and for the attenuation of muscle damage and protein breakdown in muscle. Numerous studies suggest that HMB can diminish or partly prevent exercise-induced muscle damage and/or muscle proteolysis (likely mediated by the ubiquitin proteasomal pathway—see the section on “Cellular Protein Degradation Systems”) [52–57].

Isoleucine

Transaminase or transferase
 α -keto β -methyl valerate
 NAD^+ CoA
 BCKAD^* ①
 NADH CO_2
 α -methylbutyryl CoA
Dehydrogenase
 FAD^+ FADH_2
Tiglyl CoA
 H_2O Hydratase
 α -methyl β -hydroxy butyryl CoA
 NAD^+ β -hydroxyacyl CoA dehydrogenase
 $\text{NADH} + \text{H}^+$
 α -methylacetoacetyl CoA
CoA Acetyl CoA acyl transferase
Propionyl CoA Acetyl CoA
ATP HCO_3^-
Propionyl CoA carboxylase-(biotin) ②
AMP + PP_i
D-methylmalonyl CoA
Racemase
L-methylmalonyl CoA**
Methylmalonyl CoA mutase-(vitamin B_{12}) ③
Succinyl CoA

Leucine

Transferase or transaminase
 α -ketoisopropane
 NAD^+ CoA
 BCKAD^* ①
 NADH CO_2
Isovaleryl CoA
 O_2 CO_2
 β -hydroxy- β -methylbutyrate (HMB)
HMB-CoA
 FAD^+ Isovaleryl CoA dehydrogenase
 FADH_2
 β -methylcrotonyl CoA
 HCO_3^- ATP
 β -methyl crotonyl CoA carboxylase (biotin)
 H_2O ADP + P_i
 β -methylglutaconyl CoA
 H_2O β -methylglutaconyl CoA hydratase
 β -hydroxy- β -methylglutaryl CoA (HMG CoA)
HMG CoA lyase
Acetoacetate
 CoA Thiolase
Acetoacetyl CoA
Succinyl CoA Transferase
Succinate
 CoA

Valine

Transferase or transaminase
 α -ketoisovalerate
 NAD^+ CoA
 BCKAD^* ①
 NADH CO_2
Isobutyryl CoA
 FAD^+ α -methylacyl CoA dehydrogenase
 FADH_2
Methylarylyl CoA
Hydratase
 H_2O
 β -hydroxyisobutyryl CoA
 H_2O β -hydroxyisobutyryl hydroxylase
 β -hydroxyisobutyrate
 NAD^+ β -hydroxyisobutyrate dehydrogenase
 $\text{NADH} + \text{H}^+$
Methylmalonate semialdehyde
Methylmalonic semialdehyde dehydrogenase
 CoA NAD⁺
Amino isobutyrate
L-methylmalonyl CoA**
Methylmalonyl CoA mutase-(vitamin B_{12}) ③
Succinyl CoA

*Branched-chain α -keto acid dehydrogenase (BCKAD), requiring thiamin as TDP/TPP, niacin as NADH, and Mg^{2+} and CoA from pantothenate
**Common intermediate in the catabolism of methionine, threonine, isoleucine, and valine

① Defect in this enzyme complex causes maple syrup urine disease.

② Defect in this enzyme results in propionic acidemia.

③ Defect in this enzyme results in methylmalonic acidemia.

Figure 6.37 Branched-chain amino acid metabolism.

Several defects in enzymes have been identified in leucine degradation (Figure 6.37). Defects in isovaleryl CoA dehydrogenase result in isovaleric acidemia. Defects in β -methyl crotonyl CoA carboxylase cause β -methyl crotonylglycinuria. Impaired activity of β -methylglutaconyl CoA hydratase causes β -methylglutaconic aciduria, and altered activity of β -hydroxyl β -methylglutaryl (HMG) CoA lyase causes β -hydroxyl β -methylglutaric aciduria. Each of these disorders results in the production and accumulation of numerous acids and other compounds in body fluids and a variety of other problems including convulsions, coma, acidosis, dehydration, seizures, mental retardation, and so on. A leucine-restricted diet is typically prescribed for these conditions. In some cases, to decrease toxic compounds from accumulating, supplements of carnitine and glycine may be needed. Fat restriction is also needed for those with HMG CoA lyase deficiency.

Leucine is one of the few amino acids that is completely oxidized in the muscle for energy. Leucine is oxidized in a manner similar to fatty acids, and its oxidation results in the production of 1 mol of acetyl CoA and 1 mol of acetoacetate. Complete oxidation of leucine generates more ATP molecules on a molar basis than complete oxidation of glucose. Moreover, leucine oxidation in muscles increases during certain physiological states, such as fasting. During fasting, leucine rises to high levels in the blood and muscle, while the capacity of the muscle to degrade leucine increases concurrently. By supplying the muscle with the equivalent of 3 mol of acetyl CoA per molecule of leucine oxidized, the acetyl CoA produces energy for the muscle while simultaneously inhibiting pyruvate oxidation. Pyruvate is then used for the synthesis of lactate, which is released from the muscles. Thus, the oxidation of leucine spares essential gluconeogenic precursors. Pyruvate, along with lactate, can be returned to the liver; the former is either transported as pyruvate per se or (more likely) converted to alanine for transport.

A great deal of interest in protein metabolism within the skeletal muscle was generated by the discovery that during starvation the amounts of the various amino acids released from the muscle could not reflect proteolysis alone. In particular, much more alanine and glutamine were appearing in the blood than could be attributed to muscle protein content. We now know that alanine is made in muscle from a transamination reaction with pyruvate and α -ketoglutarate. Pyruvate is generated from glucose metabolism within the muscle cells, and glutamate generates α -ketoglutarate following transamination of any of the branched-chain amino acids. The alanine then leaves the muscle for transport in the blood to the liver. During times of starvation, low carbohydrate intake (e.g., high glucagon:insulin ratio), or stress (e.g., high epinephrine and cortisol concentrations), the liver converts the alanine back to pyruvate, which through a series of reactions is converted back to glucose as part

of gluconeogenesis. Remember, this cycling of alanine and glucose between the muscle and liver is known as the alanine-glucose cycle, as discussed in the previous section and shown in Figure 6.36.

Glutamine is generated in muscle in several pathways as shown in Figure 6.38. Initially, transamination of the branched-chain amino acids occurs primarily with α -ketoglutarate to form the branched-chain α -keto acid and glutamate, respectively. Glutamate combines with ammonia to form glutamine in an ATP-dependent reaction catalyzed by glutamine synthetase. Glutamine synthetase activity is relatively high in skeletal muscle, as well as in the lungs, brain, and adipose. Ammonia may be generated by amino acid deamination or by AMP deamination. AMP forms in the muscle with ATP degradation as occurs rapidly with exercise. The glutamine that is formed in the muscle is released into the blood and transported for use by other tissues, such as the intestine, kidneys, or liver.

Amino acids released in lesser quantities from muscle (forearm and/or leg) in a postabsorptive state include phenylalanine, methionine, lysine, arginine, histidine, tyrosine, proline, tryptophan, threonine, and glycine [59,60]. Further studies investigating the effects of meals containing all three energy nutrients on amino acid uptake and output by muscle are needed.

Creatine, a nitrogen-containing compound that is made in the kidney and liver from the amino acids arginine and glycine with methyl groups donated from the amino acid methionine, functions in skeletal muscle as an energy source. Remember from earlier in this chapter that creatine is phosphorylated in muscle by ATP to form phosphocreatine (also called creatine phosphate), which can replenish ATP in muscles that are actively contracting (as with exercise). Phosphocreatine works in muscle by reacting with ADP generated in the muscle from the hydrolysis of ATP. When phosphocreatine reacts with ADP, creatine and ATP are formed. Creatine kinase catalyzes this important reaction that enables the generation of ATP for muscle contraction. Creatine and creatine phosphate, however, do not remain indefinitely in muscle; rather, both slowly but spontaneously cyclize (Figure 6.39) because of nonreversible, nonenzymatic dehydration. This cyclization of creatine and phosphocreatine forms creatinine. Once formed, creatinine leaves the muscle, passes across the glomerulus of the kidney, and is excreted in the urine. Small amounts of creatinine may be excreted into the gut and, like urea, metabolized by bacterial flora in the intestine. Creatinine clearance is frequently used as a means of estimating kidney function.

Nitrogen-Containing Compounds as Indicators of Muscle Mass and Muscle/Protein Catabolism

Urinary excretion of creatinine and 3-methylhistidine is used as indicators of the amount of existing muscle mass and the rate of muscle degradation, respectively.

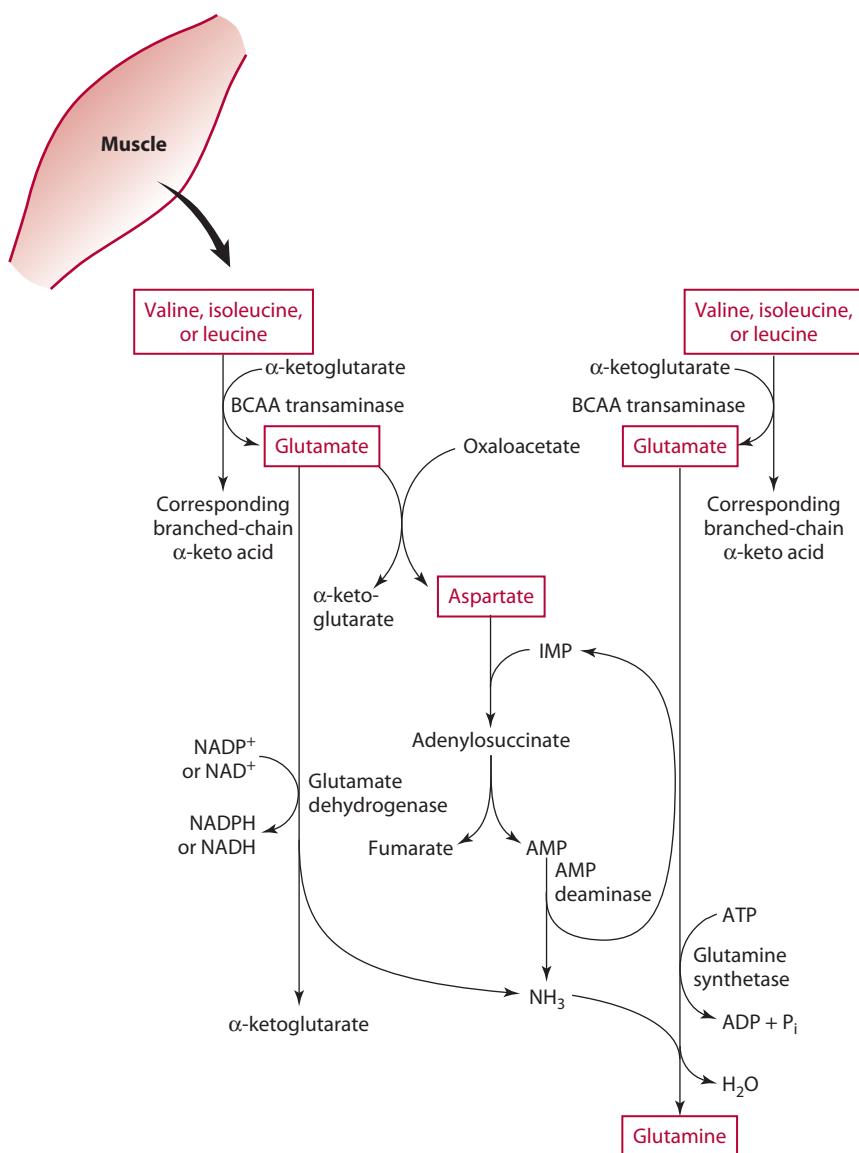


Figure 6.38 Some pathways of glutamine generation in muscle. See text page 226 for details.

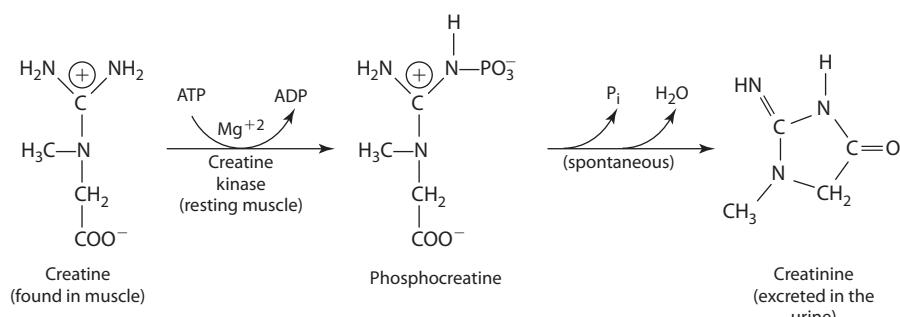


Figure 6.39 Conversion of creatine to phosphocreatine in the muscle and its spontaneous cyclization to creatinine.

Urinary creatinine excretion is considered to be a reflection of muscle mass because it is the degradation product of creatine, which makes up approximately 0.3% to 0.5% of muscle mass by weight. The creatinine excreted in the urine reflects about 1.7% of the total creatine pool per day.

However, urinary creatinine excretion is not considered to be a completely accurate indicator of muscle mass because of the variation in muscle creatine content.

Excretion of 3-methylhistidine provides an indicator of muscle catabolism (degradation). On proteolysis,

3-methylhistidine (shown in Table 6.1) is released and is a nonreusable amino acid because the methylation of histidine occurs posttranslationally. Because 3-methylhistidine is found primarily in actin, this compound has been used to estimate muscle protein degradation. However, actin is not found only in muscle but appears to be widely distributed in the body, including in tissues such as the intestine and platelets, which have high turnover rates. Thus, urinary 3-methylhistidine excretion represents not only an index of muscle breakdown but more inclusively an index of protein breakdown for many tissues in the body.

KIDNEYS

Studies in humans as well as animals suggest that the kidneys preferentially take up a number of amino acids including, for example, glycine, alanine, glutamine, glutamate, phenylalanine, and aspartate. Glutamine uptake by the kidney, for example, has been estimated at 7 to 10 g per day [61]. Metabolism of amino acids within the kidney (Figure 6.40) includes these events:

- serine synthesis from glycine
- glycine catabolism to ammonia
- histidine generation from carnosine degradation

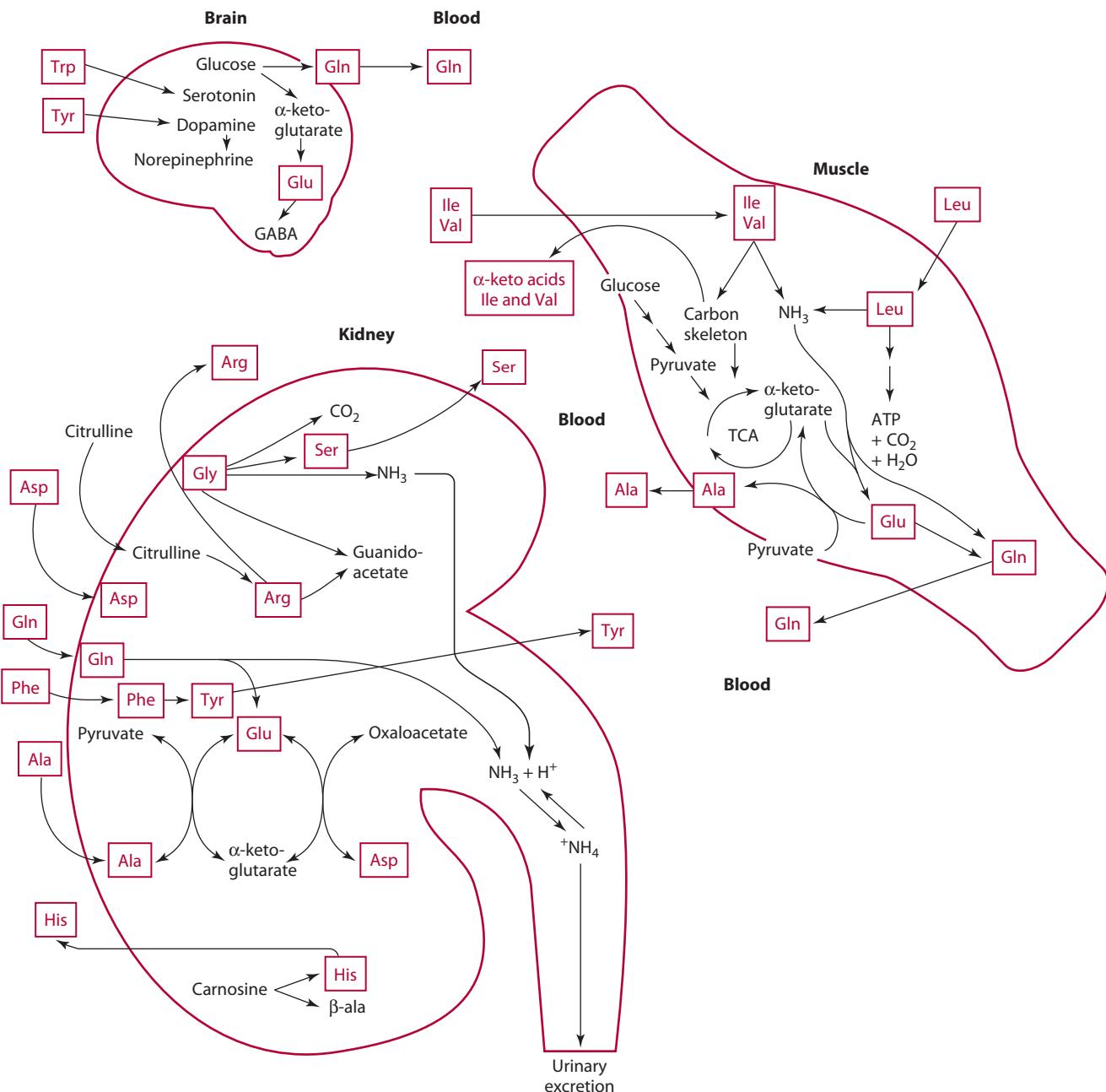


Figure 6.40 Amino acid metabolism in selected organs.

- arginine synthesis from citrulline
- tyrosine synthesis from phenylalanine
- guanidoacetate formation from arginine and glycine for creatine synthesis

The kidney is considered to be the major site for arginine, histidine, serine, and perhaps tyrosine production [61].

In addition, the kidney is the only organ besides the liver that has the enzymes necessary for gluconeogenesis. This renal metabolism of amino acids becomes particularly significant during fasting. Gluconeogenesis can raise the amount of glucose available to the body for energy, and ammonia (formed from deamination of amino acids, especially glutamine) can help normalize the blood pH, which typically decreases with fasting. Acidosis occurs with fasting because of the resulting rise in ketone concentration in the blood. Also, a loss of sodium and potassium occurs as these minerals are excreted in the urine along with the ketones. Renal glutamine uptake for ammonia production during periods of acidosis (or low bicarbonate concentrations) is increased, while uptake by the intestine, liver, and other organs is diminished. Ammonia generated from amino acids, especially glutamine deamination, enters the filtrate and combines with H⁺ ions to form ammonium ions. The ammonium ions cannot be reabsorbed and thus are excreted in the urine. The loss of the H⁺ from the body serves to increase pH from an acidotic state toward a normal value of about 7.35 to 7.45.

Arginine is thought to be synthesized in sufficient quantities by adults and is thus considered as a dispensable amino acid. Most arginine not used for urea generation is made in the body in the kidneys (rather than the liver, which makes arginine but immediately degrades it to form urea) from citrulline taken out of the blood. Arginine is used in the body for the synthesis of protein, agmatine (involved in cell signalling, proliferation, and nitric oxide regulation), polyamines (putrescine, spermine, and spermidine), and creatine. It is estimated that the kidneys extract about 1.5 g citrulline (mostly released from intestinal cells) per day and release about 2 to 4 g arginine daily [61].

Phenylalanine metabolism to tyrosine in the kidney also has been demonstrated. It is estimated that the kidney takes up about 0.5 to 1 g phenylalanine per day and releases about 1 g tyrosine [61].

Serine can also be made from glycine in the kidney (proximal tubule). The kidneys are thought to take up about 1.5 g glycine per day. Glycine, however, is also generated from glutathione catabolism in the proximal tubules of the kidneys.

The role of the kidneys in nitrogen metabolism cannot be overemphasized. The organ is responsible for ridding the body of nitrogenous wastes that have accumulated in the plasma. Moreover, enzymes particularly active in the kidney and involved in removal of nitrogenous

compounds from the body include the aminotransferases, glutamate dehydrogenase, and glutaminase, all of which catalyze the removal of ammonia from glutamate and glutamine. Kidney glomeruli act as filters of blood plasma, and all the constituents in plasma with the exception of plasma proteins move into the filtrate. Essential nutrients such as sodium (Na⁺), amino acids, and glucose are actively reabsorbed as the filtrate moves through the tubules. Many other substances are not actively reabsorbed and if they move into the tubular cells must either move along an electrical gradient or move osmotically with water. The amount of these substances that enters the tubular cells, then, depends on how much water moves into the cells and how permeable the cells are to the specific substances. The cell membranes are relatively impermeable to urea and uric acid, whereas membranes are particularly impermeable to creatinine, none of which is typically reabsorbed.

Several forms of nitrogenous wastes are lost in the urine, as shown in Table 6.7. About 80% of nitrogen is lost in the urine as urea under normal conditions. In acidotic conditions, as occur with fasting, urinary urea nitrogen losses decrease, as does the percentage of nitrogen lost as urea. Urinary ammonia excretion rises in both absolute terms and percentage terms in acidotic conditions. In addition to urea and ammonia, usual nitrogenous wastes found in the urine include creatinine and uric acid, with lesser or trace amounts of creatine (<100 mg/day), protein (<100 mg/day), amino acids (<700 mg/day), and hippuric acid (<100 mg/day). Hippuric acid results from the conjugation of the amino acid glycine and benzoic acid, which is generated mostly in the liver from aromatic compounds. Because the benzoic acid is not water soluble, it must be conjugated for excretion. Trace amounts of other nitrogen-containing compounds such as porphobilinogen and metabolites of tryptophan also may be present in the urine. In addition to urinary nitrogen losses, nitrogen may be lost through the skin as urea. Nitrogen also may be lost with the loss of hair and skin cells. These losses are referred to as insensible nitrogen losses.

BRAIN AND ACCESSORY TISSUES

The brain has a high capacity for the active transport of amino acids. In fact, the brain has transport systems for neutral, dibasic, and dicarboxylic amino acids. The

Table 6.7 Nitrogen-Containing Waste Products Excreted in the Urine

Compound	Approximate Amount Excreted/Day g/day	mmol/N
Urea	5–20	162–650
Creatinine	0.6–1.8	16–50
Uric acid	0.2–1.0	4–20
Ammonia	0.4–1.5	22–83

transporters for some of the amino acids are almost fully saturated at normal plasma concentrations; this is especially true of the transporters for the large neutral amino acids like the branched-chain amino acids and the aromatic amino acids, which can compete with each other for the common carriers. The effects of this competition become especially apparent in conditions in which the blood concentrations of any of the branched-chain or aromatic amino acids become elevated. For example, in PKU, elevations in blood phenylalanine result in increased uptake of this amino acid by the brain. In MSUD, elevations in blood leucine, isoleucine, and valine result in the increased uptake of branched-chain amino acids (at the expense of the aromatic amino acids) into the brain. Moreover, in liver disease for example, the concentrations of the aromatic amino acids exceed those of the branched-chain amino acids and cause increase uptake of the aromatic amino acids by the brain. The elevations of these amino acids in the brain alter brain function causing a variety of neurologic problems such as impaired brain development and altered behavior and mental function, among other manifestations.

Biogenic Amines and Neurotransmitters/Hormones

The uptake of two aromatic amino acids, tryptophan and tyrosine, by the brain is particularly important because

these amino acids act as precursors for a variety of hormones and/or neurotransmitters or modulators of nerve function.

- Tryptophan is used in the synthesis of the hormone melatonin (N-acetyl 5-methoxyserotonin) and the neurotransmitter serotonin (5-hydroxytryptamine; Figure 6.41). Melatonin is made primarily in the pineal gland, which lies in the center of the brain. Melatonin synthesis and release correspond with darkness; the hormone is thought to be involved mainly with the regulation of circadian rhythms and sleep, and supplement use has been helpful for some people with jet lag [62–64]. Serotonin functions as an excitatory neurotransmitter and as a potent vasoconstrictor and stimulator of smooth muscle contraction.
- Tyrosine is used to synthesize (mostly in the adrenal medulla) dopamine, norepinephrine, and epinephrine (Figure 6.28), called catecholamines because they are derivatives of catechol (Figure 6.42) or amines. The actions of both epinephrine and norepinephrine have major effects on nutrient metabolism. These actions include stimulation of glycogenolysis and lipolysis (mobilization of fatty acids), and increased metabolic rate, among other effects.

Neurotransmitters are stored in the nerve axon terminal as vesicles or granules until stimuli arrive to effect their

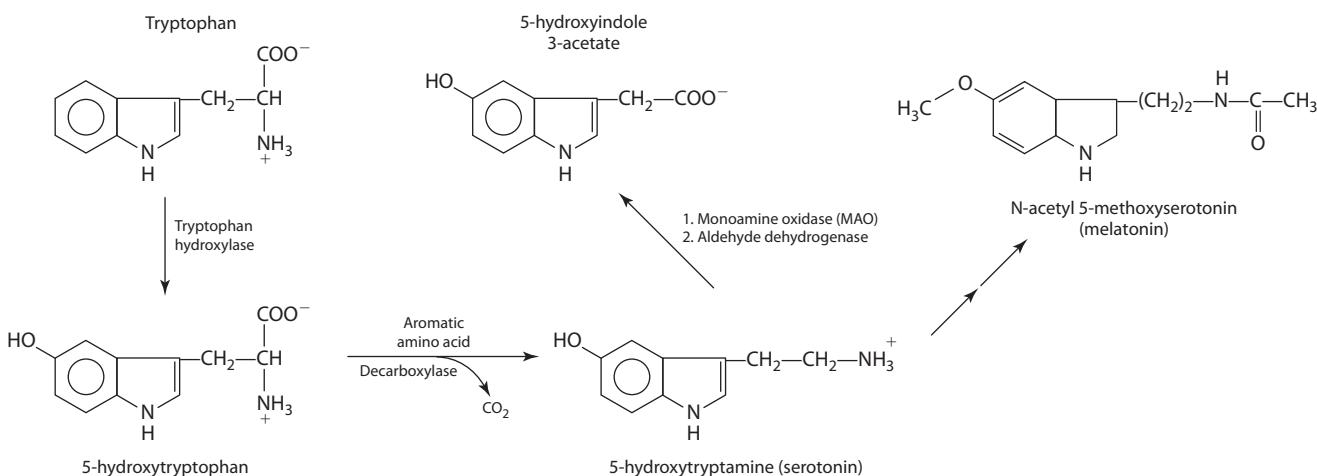


Figure 6.41 Serotonin and melatonin synthesis and degradation from tryptophan.

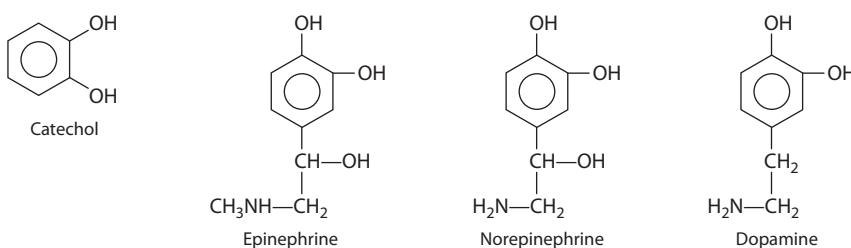


Figure 6.42 The catecholamines.

release. Following their action on the cell membranes, the neurotransmitters are inactivated. The fastest mechanism for inactivation is uptake of the neurotransmitter by adjacent cells, where mitochondrial monoamine oxidase (MAO) removes the amine group. Each of the catecholamines (dopamine, norepinephrine, epinephrine) as well as serotonin can be inactivated by MAO. A slower mechanism of inactivation requires that the catecholamines be carried by the blood to the liver, where they are methylated by catechol-O-methyltransferase (COMT). Interactions between MAO inhibitors and foods high in amines such as tyramine are discussed in the Chapter 12 Perspective on nutrient-drug interactions.

Other amino acids besides tryptophan and tyrosine also function as neurotransmitters in the brain:

- Glycine acts as an inhibitory neurotransmitter.
- Taurine is also thought to function as an inhibitory neurotransmitter.
- Aspartate, derived chiefly from glutamate through aspartate aminotransferase activity common in neural tissue, is thought to act as an excitatory neurotransmitter in the central nervous system.
- Glutamate acts as an excitatory neurotransmitter or may be converted into γ -amino butyric acid (GABA), an inhibitory neurotransmitter.

GABA is believed to be the neurotransmitter for cells that exert inhibitory effects on other cells in the central nervous system. The conversion of glutamate to GABA involves the removal of the α -carboxyl group of glutamate by the enzyme glutamate decarboxylase in a vitamin B₆-(PLP)-dependent reaction (Figure 6.43). Glutamate uptake by the brain typically is low; thus, synthesis of glutamate from glucose represents the primary source of brain glutamate.

Glutamate is of significance to the brain not just as a neuromodulator or a precursor of GABA but also as a means of ridding the brain of ammonia. Little glutamate is transported into the brain from the blood. The starting point for glutamate metabolism is the synthesis of α -ketoglutarate from glucose that has been transported from the blood across the blood-brain barrier. The α -ketoglutarate can be converted to glutamate through reductive amination.

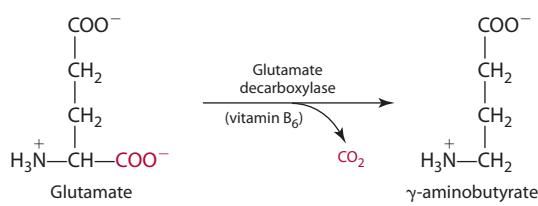


Figure 6.43 GABA synthesis.

Whenever excessive ammonia is present in the brain, glutamine is formed through the action of glutamine synthetase, which is highly active in neural tissues (Figure 6.35). A glutamate-glutamine cycle in the brain is thought to function as follows. Neurons take up glutamine and convert it to glutamate using glutaminase. The glutamate is released into the synapse (extracellular fluid) and then is taken up by astrocytes. Astrocytes convert the glutamate back to glutamine. The glutamine is then released. It can be reused by the neuron, but it is also freely diffusible and can move easily into the blood or cerebrospinal fluid, thereby allowing the removal of 2 mol of toxic ammonia from the brain. Any condition that causes an unusual elevation of blood ammonia can interfere with the normal handling of amino acids by the brain. The goal of treating hepatic encephalopathy (dysfunction of the brain associated with liver disease, which characteristically results in elevated blood ammonia concentrations) is to normalize the effects of altered amino acid metabolism on the central nervous system.

Leucine as well as the other branched-chain amino acids provides the brain with nitrogen (amino groups). Astrocytes are thought to initially take up leucine and using aminotransferases remove its amino group for synthesis of glutamate and glutamine. Leucine's α -ketoacid (α -ketoisocaproate), which is synthesized when the amino group is removed, is then taken up by neurons and reaminated to leucine using glutamate. Leucine and the other branched-chain amino acids are thought to provide 30% to 50% of the amino groups used by the brain for glutamate synthesis [65].

Use of amino acid supplements has been promoted with claims that, upon ingestion, the amino acids are used in the body to synthesize compounds necessary to evoke the desired response. For example, tryptophan supplements have been promoted to induce sleep. However, use of tryptophan supplements as sleep aids has not proven effective.

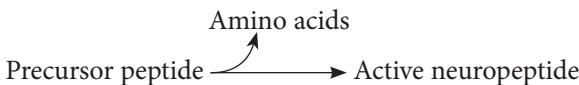
Neuropeptides

The central nervous system abounds in peptides, termed neuropeptides. Many of the same peptides that were mentioned in Chapter 2 with respect to digestion and were found associated with the intestinal tract are also found associated with the central nervous system. These neuropeptides are of varying lengths and possess a variety of functions. Some peptides act as hormone-releasing factors, such as ACTH involved with cortisol release. Some have endocrine effects, such as somatotropin or growth hormone. Others have modulatory actions on transmitter functions, mood, or behavior, such as the enkephalins. The enkephalins and endorphins, though similar to natural opiates, possess a wide range of functions, including affecting pain sensation, blood pressure and body temperature regulation, governance of body movement, secretion of

hormones, control of feeding, and modulation of learning ability.

The neurosecretory cells of the hypothalamus are foremost in the secretion of the neuropeptides. Those that have hormone action move out of the axons of the nerve cells into the pituitary, from which they are secreted. This linkage between the nervous system and the pituitary is of great significance in the overall control of metabolism because the pituitary gland is primary in coordinating the various endocrine glands scattered throughout the body.

The neuropeptides are believed to be synthesized from their constituent amino acids via the DNA coding, messenger RNA (mRNA), ribosomes, and transfer RNA (tRNA) system. Because the nucleus and ribosomes are found in the cell body and dendrites, the peptides must travel to the end of the axon to be stored in vesicles for future release. The neuropeptides are stored as inactive precursor polypeptides, which must be cleaved to generate an active neuropeptide, as shown here:



Following synthesis of the active neuropeptide, it is released by exocytosis to perform its function at the membrane. After performing its function, the neuropeptide is hydrolyzed to its constituent amino acids.

Protein Turnover: Synthesis and Catabolism of Tissue Proteins

Food intake and the nutritional status of the organism affect protein turnover that is mediated through changes in the concentrations of different hormones. The secretion of insulin, glucagon, growth hormone, and glucocorticoids increases in response to elevated concentrations of selected amino acids. In general, increased protein synthesis, decreased protein degradation, and positive nitrogen balance are promoted by insulin, whereas the counterregulatory hormones, glucagon, epinephrine, and glucocorticoids have an opposite effect, promoting overall protein degradation and a negative nitrogen balance. Growth hormone, though counterregulatory, is anabolic, like insulin. Prostaglandins and thyroid hormones also are affected by dietary nutrient intakes and can promote changes in protein turnover. Nitric oxide has been shown to inhibit hepatic protein synthesis [66]. The effects of a hormone on protein turnover, however, may differ depending on the tissue. For example, a glucagon:insulin ratio favoring glucagon diminishes insulin's ability to inhibit protein degradation and diminishes the overall rate of protein

synthesis, yet it stimulates hepatic synthesis of proteins (enzymes) for gluconeogenesis and ureagenesis.

Each cellular protein exhibits a specific and characteristic rate of synthesis. Transcription and translation of DNA and RNA are under multiple influences, such as hormonal and nutrient. Protein synthesis is also affected by the amount and stability of mRNA, the ribosome number (amount of ribosomal RNA, or rRNA), the activity of the ribosomes (rapidity of translation, or peptide formation), the presence of both essential and nonessential amino acids in the appropriate concentrations to charge the tRNA, and the hormonal environment. Should amino acids not be present or be present in insufficient quantity, amino acid oxidation increases. In other words, the supply of amino acids must meet the demand. The rate of amino acid oxidation is sensitive to a surplus or deficit of specific amino acids as well as to hormonal factors and thus regulates the amino acid pool(s). Amino acid pools remain fairly constant in size and pattern in the body. Further, they serve as the connecting link between two cycles of nitrogen metabolism:

protein turnover (protein synthesis vs. degradation)	and	nitrogen balance (nitrogen intake vs. nitrogen output)
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These two cycles operate somewhat independently, but if either cycle gets out of balance, the other is affected to some degree. For example, during growth, protein synthesis exceeds degradation, and nitrogen intake exceeds excretion, resulting in a positive nitrogen balance. The increase in protein turnover, however, is much greater than is reflected by the change in nitrogen balance. The amino acid pool(s), regulated in some manner, act as balast between protein turnover and nitrogen balance.

When the body is considered as a whole, protein synthesis and degradation (turnover) are estimated to account for 10% to 25% of resting energy expenditure [67]. Protein degradation, for example, requires energy for proteolysis, metabolism of amino acids not reincorporated into protein, amino acid transport across membranes, and RNA metabolism [67].

Protein synthesis and protein degradation are under independent controls. Rates of synthesis can be quite high, as with protein accretion during growth. Alternately, protein degradation can be quite high, as during fever. Rates of protein turnover vary among tissues, as is evidenced in the more rapid turnover of visceral protein as compared with skeletal muscle. Because of its mass, muscle accounts for about 25% to 35% of all protein turnover in the body.

CELLULAR PROTEIN DEGRADATION SYSTEMS

Degradation of proteins, either made intracellularly or brought into the cell by endocytosis, occurs primarily by the action of proteases, either lysosomal or nonlysosomal (proteosomal), present in the cytosol. The contributions

of the pathways in proteolysis vary depending on the cell type and the physiological status [68]. Nonetheless, the constant degradation of intracellular proteins is of prime importance to the life of the cells because it ensures a flux of proteins (amino acids) through the cytosol that can be used for cellular growth and/or maintenance.

Lysosomal Degradation

Autophagic (“self-eating”) proteolysis by lysosomes involves three main steps: sequestration, fusion and acidification, and digestion. During sequestration, a double-layered membrane is created that encloses a part of the extracellular contents and the cell cytosol and its contents, forming a vacuole or early autophagosome. Next, the autophagosome fuses with a lysosome and is acidified by the action of proton pumps, creating what is called a late autophagosome. Lysosomes are cell organelles that contain a variety of digestive enzymes and help to degrade (turn over) various cell parts. Lysosomes are found in all mammalian cell types with the exception of the erythrocyte, but to varying degrees. For example, although skeletal muscles do not contain many lysosomes, lysosomes are particularly abundant in liver cells. Several proteases are found in lysosomes, including endopeptidases and exopeptidases known as cathepsins. Numerous cathepsins have been isolated; examples include protease cathepsins B, H, and L, which are called cysteine proteases, and protease cathepsin D, which is an aspartate protease. The cathepsin proteases vary in specificity [69]. Together, the proteases and other lysosomal enzymes digest cellular proteins and components. No energy is needed. The amino acids released from cellular proteolysis can be reused by the cell.

Autophagic proteolysis by lysosomal proteases is thought to be responsible for degradation of membrane-associated proteins, extracellular proteins (especially asialoglycoproteins that have lost a sialic acid moiety off one end) brought into the cell by endocytosis, and longer-lived intracellular proteins under conditions of nutrient deprivation and

some pathological conditions [68,69]. Autophagy in liver cells is enhanced by glucagon and suppressed by insulin and by amino acids [70]. The high glucagon:insulin ratio is consistent with conditions in which cells are nutritionally (amino acid) deprived. The extent to which lysosomal proteases influence muscle proteolysis is unknown, but it is believed to be fairly small [71]. In liver cells, cellular uptake and accumulation of three amino acids, leucine, phenylalanine, and tyrosine, and increases in cell volume associated with the sodium-dependent cellular uptake of alanine, glutamine, and proline, are known to inhibit autophagy. The exact mechanisms by which amino acid uptake inhibits proteolysis are not known.

Proteasomal Degradation

In addition to lysosome-mediated cellular protein degradation, nonlysosomal protease systems or complexes (called proteasomes) also degrade proteins. The ubiquitin proteasomal system is nonlysosomal and mediates the degradation of many cellular proteins. The proteasome is a large, oligomeric structure with a cavity where protein degradation occurs. In the ubiquitin system, proteins that are to be degraded are ligated to **ubiquitin** (a 76-amino-acid polypeptide) in an ATP-requiring reaction as shown in Figure 6.44. Before it can be linked to a protein, ubiquitin must be activated. Ubiquitin is activated by the enzyme E1. E1 is a subunit of the ubiquitin enzyme system, which hydrolyzes ATP to form a thiol ester with the carboxyl end of ubiquitin. This activated ubiquitin is transferred to another enzyme protein, E2. Next, the carboxyl end of ubiquitin is ligated by E3 to the protein substrate that is ultimately to be degraded. One or more (typically five) ubiquitin proteins may bind to a protein substrate. ATP is required to unfold the tertiary and secondary structure of the proteins. Once ubiquitins are ligated to the protein to be degraded and the protein structure permits, proteases present as a proteasome/multienzyme complex degrade the ubiquitinated proteins in a

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series of reactions. Complexes of cap proteins regulate the reactions of the proteasome complex. Following proteolysis, ubiquitin is released for reuse.

Proteasomal degradation is thought to be responsible for the degradation of abnormal, damaged, denatured, or mislocated proteins and of regulatory proteins that typically have short half-lives (often less than 30 minutes) [68,72]. Activity of ubiquitin-dependent proteasomal degradation also appears to increase during pathological conditions such as sepsis, cancer, and trauma, among others, as well as during starvation [71,73]. Cytokines are thought to be involved (in part) with its activation. Alternately, a metabolite of leucine, β -hydroxy β -methylbutyrate (HMB) is thought to attenuate protein degradation by the proteasomal pathway, possibly by phosphorylating (inactivating) kinases that are involved in the expression of the proteasome [54].

One signal for ubiquitin-mediated proteasomal degradation in proteins involves N-terminal (N-end rule) recognition by the E3 α ubiquitin protein ligase [74]. E3 has two distinct sites that interact with specific N-terminal residues. Proteins with valine, methionine, glycine, alanine, serine, threonine, and cysteine residues at their N-terminal position are relatively stable [75]. In contrast, proteins with N-terminal basic or large hydrophobic amino acids such as lysine, arginine, histidine, leucine, isoleucine, asparagine, glutamine, tryptophan, phenylalanine, and tyrosine are typically susceptible to degradation by the ubiquitin proteasomal system [74,75]. Proteins with acetylated N-termini are not degraded by the ubiquitin system [74,75].

Other characteristics of short-lived proteins include large size, acidic net charge, hydrophobicity, and rapid inactivation by a low pH or a high temperature [69]. Rapidly degraded proteins may also possess a common amino acid sequence; the PEST hypothesis suggests that proteins with particularly short half-lives contain regions rich in proline (P), glutamic acid (E), serine (S), and threonine (T) [76]. However, neither the mechanism by which the PEST signal targets proteins for degradation nor the system that recognizes the signal has been identified [71,74,76].

Calpain or Calcium-Activated Proteolytic Degradation

In addition to the ubiquitin/proteasomal and the lysosomal pathways, another degradation pathway has been identified in muscle: the calpain or calcium-activated protease/proteolytic pathway. This pathway does not require lysosomes or energy but instead requires calcium for its function. Two proteases, μ -calpain and m-calpain, comprise the calpain proteolytic pathway and differ in their need for calcium. The overall role of the calpains in protein turnover is unclear; however, the proteases may be responsible for the initial step in degradation, the release of myofilaments from the myofibrils. The released

myofilament is then thought to be ligated to ubiquitin for further degradation.

Changes in Body Mass with Age

Reference figures, first developed back in the 1970s, provide information on body composition, including muscle mass, based on average physical dimensions from measurements of thousands of people who participated in various anthropometric and nutrition surveys [77]. As seen in Table 6.8, the reference man weighs 29 lb more than the woman (nonpregnant) and is 4 in. taller. Note that in the reference man, muscle accounts for 44.8% body weight, whereas it is only 36.0% body weight in the female. The man has 15% body fat, compared with the female's 27%. Of the 15% total body fat in the reference man, only 3% is essential fat, compared with 12% essential fat in the reference woman's 27% total body fat. Essential fat is fat associated with bone marrow, the central nervous system, viscera (internal organs), and cell membranes. In females, essential fat also includes fat in mammary glands and the pelvic region. The average body densities of the reference man and woman are 1.070 and 1.040 g/mL, respectively.

Body composition is influenced by a variety of factors, including age, gender, race, heredity, and stature [78]. The influence of gender on body composition appears to exist from birth but becomes dramatically evident at puberty and continues throughout life. The effect of maturation on body composition from birth to 10 years of age has been estimated through the use of "reference infants and children"

Table 6.8 Body Composition of Reference Man and Woman

Reference Man	Reference Woman
Age: 20–24 yr	Age: 20–24 yr
Height: 68.5 in.	Height: 64.5 in.
Weight: 154 lb	Weight: 125 lb
Total fat: 23.1 lb (15.0% body weight)	Total fat: 33.8 lb (27.0% body weight)
Storage fat: 18.5 lb (12.0% body weight)	Storage fat: 18.8 lb (15.0% body weight)
Essential fat: 4.6 lb (3.0% body weight)	Essential fat: 15.0 lb (12.0% body weight)
Muscle: 69 lb (44.8% body weight)	Muscle: 45 lb (36.0% body weight)
Bone: 23 lb (14.9% body weight)	Bone: 15 lb (12.0% body weight)
Remainder: 38.9 lb (25.3% body weight)	Remainder: 31.2 lb (25.0% body weight)
Average body density: 1.070 g/mL	Average body density: 1.040 g/mL

Source: From McArdle, W.D., Katch, F.I., Katch, V.L., *Exercise Physiology*, p. 369 (Philadelphia: Lea & Febiger, 1981).

(Table 6.9) [79,80]. Table 6.10 describes the components of weight gain occurring during these years.

Throughout childhood, lean body mass increases. Total body water decreases during the first year of life primarily because of a rapid increase in fat, then increases slightly from about 1 year to 6 or 7 years, and then gradually declines again for the next 3 to 4 years [79]. By age 10 years, however, total body water still exceeds 60% of total body weight. Accompanying the changes in total body water is a change in the ratio of extracellular fluid (ECF) to intracellular fluid (ICF). During infancy and childhood the ECF: ICF ratio progressively falls (because of growth and lean body mass maturation) until ICF occupies the majority position [78]. Cell hypertrophy and bone development encroach on the space occupied by ECF, whereas protein

accrual results in an increased incorporation of ICF. The percentage of water in lean body mass of reference children exceeds that considered average for the adult. In addition, the value for minerals as a percentage of body weight is less than the adult average of 5.2% [81]. These differences in the components of lean body mass in children and adults result in a density of lean body mass in children of <1.10 g/mL, the average density for adults.

Although some gender differences in the body composition of prepubescent children (Tables 6.9 and 6.10) are evident, they are not of great magnitude. The significant gender differences occur during adolescence and, once established, persist throughout adulthood. Lean body mass is the body component most significantly affected by gender. In both sexes, serum testosterone levels rise during adolescence.

Table 6.9 Body Composition of Reference Children

Age	Weight (kg)		Fat (%)		Protein (%)		Water (%)		Ash (%)		Carbohydrate (%)	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Birth	3.545	3.325	13.7	14.9	12.9	12.8	69.6	68.6	3.2	3.2	0.5	0.5
4 mo	7.060	6.300	24.7	25.2	11.9	11.9	60.1	59.6	2.8	2.8	0.4	0.4
6 mo	8.030	7.250	25.4	26.4	12.0	12.0	59.4	58.4	2.8	2.7	0.4	0.4
12 mo	10.15	9.18	22.5	23.7	12.9	12.9	61.2	60.1	2.9	2.8	0.5	0.5
18 mo	11.47	10.78	20.8	21.8	13.5	13.5	62.2	61.3	3.1	3.0	0.5	0.5
24 mo	12.59	11.91	19.5	20.4	14.0	13.9	62.9	62.2	3.2	3.0	0.5	0.5
3 yr	14.675	14.10	17.5	18.5	14.7	14.4	63.9	63.5	3.4	3.1	0.5	0.5
4 yr	16.69	15.96	15.9	17.3	15.3	14.8	64.8	64.3	3.5	3.1	0.5	0.5
5 yr	18.67	17.66	14.6	16.7	15.8	15.0	65.4	64.6	3.7	3.1	0.5	0.5
6 yr	20.69	19.52	13.5	16.4	16.2	15.2	66.0	64.7	3.8	3.2	0.5	0.5
7 yr	22.85	21.84	12.8	16.8	16.5	15.2	66.2	64.4	3.9	3.1	0.5	0.5
8 yr	25.30	24.84	13.0	17.4	16.6	15.2	65.8	63.8	4.0	3.1	0.5	0.5
9 yr	28.13	28.46	13.2	18.3	16.8	15.1	65.4	63.0	4.1	3.1	0.5	0.5
10 yr	31.44	32.55	13.7	19.4	16.8	15.0	64.8	62.0	4.1	3.1	0.5	0.5

Source: Adapted from Foman, Haschke, Ziegler, Nelson, "Body composition of reference children from birth to age 10 years." *American Journal of Clinical Nutrition*, 1982; 35:1171. © American Society for Clinical Nutrition. Reprinted by permission.

Table 6.10 Weight Increase and Its Components in Reference Children

Age	Weight Increase (g/d)		Fat (%)		Protein (%)		Minerals (%)	
	Males	Females	Males	Females	Males	Females	Males	Females
0–1 mo	29.3	26.0	20.4	21.4	12.5	12.5	0.9	0.8
3–4 mo	20.8	18.6	39.6	39.3	10.9	11.3	0.5	0.4
5–6 mo	15.2	15.0	27.3	32.4	13.2	12.6	0.4	0.4
9–12 mo	10.7	10.0	9.0	11.9	17.0	16.7	0.4	0.3
12–18 mo	7.2	8.7	7.2	10.7	18.4	17.0	0.3	0.3
18–24 mo	6.1	6.2	6.6	7.8	18.7	17.5	0.3	0.2
2–3 yr	5.7	6.0	5.8	7.9	19.1	17.6	0.2	0.2
3–4 yr	5.5	5.1	4.0	8.1	19.7	17.5	0.3	0.2
4–5 yr	5.4	4.7	3.2	11.3	19.9	17.0	0.3	0.2
5–6 yr	5.5	5.1	3.7	13.9	19.8	16.6	0.3	0.2
6–7 yr	5.9	6.4	6.3	19.6	19.5	15.6	0.3	0.2
7–8 yr	6.7	8.2	14.8	21.9	17.9	15.2	0.3	0.2
8–9 yr	7.8	9.9	15.2	24.5	17.9	14.8	0.4	0.3
9–10 yr	9.1	11.2	18.0	27.2	17.5	14.3	0.4	0.3

Source: Adapted from Foman, Haschke, Ziegler, Nelson, "Body composition of reference children from birth to age 10 years." *American Journal of Clinical Nutrition*, 1982; 35:1171. © American Society for Clinical Nutrition. Reprinted by permission.

However, the rise is much greater in boys, whose testosterone values approach ten times those of girls. As a result of high testosterone production, boys increase their lean body mass by ~33 to 35 kg between the ages of 10 and 20 years. The increment in girls is only about half as much, ~16 to 18 kg [78,82]. The female achieves her maximum lean body mass by about age 18 years, whereas the male continues accretion of lean body mass until about age 20 years [78,82]. By age 15 years the male-to-female ratio of lean body mass is 1.23:1, and by 20 years the ratio has increased to 1.45:1, well above the ratio for body weight (1.25:1) and stature (1.08:1). The pronounced gender difference in lean body mass is the primary reason for the gender difference in nutrition requirements [78].

The sharp increase in lean body mass that occurs in boys during the adolescent growth spurt is accompanied

by a decrease in the percentage of body fat. The average percentage of body fat in boys age 6 to 8 years is ~13% to 15%; this percentage decreases to 10% to 12% for boys age 14 to 16 years [83]. Although the adolescent female also increases lean body mass during the growth spurt, a higher percentage of the weight gain is caused by accretion of essential sex-specific fat. Girls age 6 to 8 years have ~16% to 18% body fat, and by age 14 to 16 years percentage of body fat ranges from 21% to 23% [83].

After 25 years of age, weight gain is usually caused by body fat accretion. Tables 6.11 and 6.12 illustrate the differences in body composition in adults of various ages. More marked changes occur with aging in females than in males; however, in both males and females, lean body mass decreases (due primarily to a decrease in protein synthesis and body cell mass) with aging [84]. Skeletal muscle

Table 6.11 Mean Body Composition Values for Healthy Males and Females*

Age (yr)	n	Weight (kg)	Protein (kg)	TBW (L)	BA (kg)	BCM (kg)	ECW (L)	ECS (kg)	TBF1 (kg)	TBF2 (kg)
Males:										
20–29	12	80.1	13.1	44.9	3.53	35.7	19.8	6.77	18.6	16.8
30–39	12	73.7	11.2	42.3	3.44	32.5	19.0	6.61	16.8	15.6
40–49	12	84.6	12.4	47.2	3.59	35.1	19.8	6.88	21.4	23.0
50–59	12	82.0	12.1	45.0	3.43	33.3	19.3	6.58	21.5	20.7
60–69	10	78.5	11.8	42.1	3.33	30.7	19.9	6.39	21.3	21.5
70–79	10	80.5	11.1	40.4	3.17	27.6	20.3	6.08	25.8	26.5
Females:										
20–29	17	64.6	9.0	33.3	2.78	23.1	16.1	5.33	19.4	20.0
30–39	10	69.3	9.3	33.6	2.69	22.9	16.5	5.17	23.7	24.7
40–49	11	65.2	8.7	31.4	2.60	21.3	15.9	4.99	22.5	22.9
50–59	9	73.6	8.4	31.7	2.40	20.5	15.9	4.61	29.1	22.6
60–69	13	61.7	7.8	28.6	2.26	18.0	15.4	4.34	23.0	23.9
70–79	9	58.3	7.3	27.6	2.03	17.6	14.5	3.89	21.4	22.3

*Protein = $6.25 \times$ total body nitrogen; BCM (body cell mass) = $0.235 \times$ total body potassium (g); ICW (intracellular water) = TBW (total body water) – ECW (extracellular water); BA (bone ash) = total body calcium (TBCa)/0.34; ECS (extracellular solids) = TBCa (total body calcium)/0.177; TBF (total body fat); TBF1 = body weight – (protein + TBW + BA); TBF2 = body weight \times (BCM + ECW + ECS).

Source: Modified from Cohn, S.H., Vaswani, A.N., Yasumura, S. et al. "Improved models for determination of body fat by in vivo neutron activation," *American Journal of Clinical Nutrition*, 1984; 40:255. © American Society for Clinical Nutrition. Reprinted by permission.

Table 6.12 Body Composition in Young and Elderly Males and Females

Parameters	Males		Females	
	Young (n = 10) (18–23 yr)	Elderly (n = 20) (61–89 yr)	Young (n = 10) (18–23 yr)	Elderly (n = 20) (60–89 yr)
Body weight (kg)	72.60 ± 10.34	61.60 ± 10.40	54.90 ± 2.71	56.10 ± 9.21
Body water (mL/kg)	586.1 ± 64.3	579.9 ± 54.4	531.0 ± 89.9	442.3 ± 57.3
Intracellular volume (mL/kg)	378.2 ± 42.3	375.7 ± 41.8	328.9 ± 64.7	248.3 ± 26.3
Lean body mass (g/kg)	800.7 ± 87.9	790.3 ± 78.1	725.4 ± 112.9	603.6 ± 77.8
Total body fat (g/kg)	199.3 ± 87.9	212.7 ± 66.2	274.6 ± 112.9	396.4 ± 77.8
Extracellular water volume (mL/kg)	207.9 ± 30.8	204.2 ± 37.0	192.1 ± 31.0	192.6 ± 19.4
Interstitial water volume (mL/kg)	167.7 ± 20.8	157.2 ± 19.7	172.5 ± 41.5	148.9 ± 21.2
Plasma volume (mL/kg)	43.2 ± 7.6	47.0 ± 6.2	29.6 ± 5.2	45.1 ± 10.1

Source: Adapted from Fülop, T., et al, "Body composition in elderly people," *Gerontology* 1985;31:150–157. Used by permission of S. Karger AG, Basel.

loss may be due in part to decreased physical activity and alterations in protein metabolism that might be affected by diminished anabolic hormone concentrations [84]. Decreased lean body mass causes a decrease in total body water. The decrease in total body water is much greater in females than in males. Further examination of body water amounts shows that extracellular fluid volume remains virtually unchanged. However, a redistribution occurs: interstitial fluid decreases while plasma volume increases [85,86]. Loss of bone mass and atrophy of organs also occur with aging.

Protein Quality and Protein Intake

Dietary protein is required by humans because it contributes to the body's supply of indispensable amino acids and to its supply of nitrogen for the synthesis of the dispensable amino acids. The quality of a protein depends to some extent on its digestibility but primarily on its indispensable amino acid composition, both the specific amounts and the proportions of these amino acids. Protein-containing foods can be divided into two categories:

- high-quality or complete proteins
- low-quality or incomplete proteins

A **complete protein** contains all the indispensable amino acids in the approximate amounts needed by humans. Sources of complete proteins are mostly foods of animal origin such as milk, yogurt, cheese, eggs, meat, fish, and poultry. The exceptions are gelatin, which is of animal origin but does not have the indispensable amino acid tryptophan, and soy protein, which is of plant origin but is a complete protein. **Incomplete proteins** or low-quality proteins are derived from plant foods such as legumes, vegetables, cereals, and grain products. Most plant foods tend to have too little of one or more particular indispensable amino acids. The term **limiting amino acid** is used to describe the indispensable amino acid that is present in the lowest quantity in the food. Listed in Table 6.13 are examples of incomplete protein containing foods and their limiting amino acid(s).

Unless carefully planned, a diet containing only low-quality proteins results in inadequate availability of selected amino acids and inhibits the body's ability to synthesize its own body proteins. The body cannot make a protein with a missing amino acid.

Table 6.13

Food Source of Incomplete Protein	Limiting Amino Acid(s)
Wheat, rice, corn, other grains and grain products	Lysine, threonine (sometimes), and tryptophan (sometimes)
Legumes	Methionine

To ensure that the body receives all the indispensable amino acids, certain proteins can be ingested together or combined so that their amino acid patterns become complementary. This practice or strategy is called mutual supplementation. For example, legumes, with their high content of lysine but low content of sulfur-containing amino acids, complement the grains, which are more than adequate in methionine and cysteine but limited in lysine. The lacto-ovo vegetarian should have no problem with protein adequacy because when milk and eggs are combined—even in small amounts—with plant foods, the indispensable amino acids are supplied in adequate amounts. One exception is the combination of milk with legumes. Although milk contains more methionine and cysteine per gram of protein than do the legumes, it still fails to meet the standard of the ideal pattern for the sulfur-containing amino acids.

Digestibility of proteins is also important for amino acid use. The digestibility of a protein is a measure of the amounts of amino acids that are absorbed following ingestion of a given protein. Animal proteins have been found to be about 90% to 99% digestible, whereas plant proteins are about 70% to 90% digestible. Meat and cheese, for example, have a digestibility of 95%, and eggs are 97% digestible. Cooked split peas are about 70% digestible, and tofu is about 90% digestible. Both the digestibility of a protein and its amino acid content affect protein quality.

EVALUATION OF PROTEIN QUALITY

Several methods are available to determine the protein quality of foods and to assess the protein adequacy of the diet. A few of these methods are discussed in this section.

Nitrogen Balance/Nitrogen Status

Nitrogen balance studies involve the evaluation of dietary nitrogen intake and the measurement and summation of nitrogen losses from the body. Nitrogen balance studies can be conducted when subjects consume a diet with a protein (nitrogen) intake that is at or near a predicted adequate amount, less than (including protein-free nitrogen) a predicted adequate amount, or greater than a predicted adequate amount. The technique or modified versions of the technique are often done with hospitalized patients to determine whether protein intake is adequate.

To determine nitrogen balance or status, nitrogen intake and output must be assessed. Assessment of nitrogen intake is based on protein intake. Protein contains approximately 16% nitrogen. Thus, to calculate grams of nitrogen consumed from grams of protein, we can do the following calculation: $0.16 \times \text{protein ingested} (\text{measured in grams}) = \text{nitrogen} (\text{measured in grams})$. Expressed alternately, ingested protein (g)/6.25 = ingested nitrogen (g). So, for example,

a 70 g protein intake provides 11.2 g nitrogen. To reverse the calculations and convert grams nitrogen into grams of protein: protein (g) = nitrogen (g) × 100/16, or protein (g) = nitrogen (g) × 6.25.

Nitrogen losses are measured in the urine (U), feces (F), and skin (S). For example, in the urine, nitrogen is found mainly as urea but also as creatinine, amino acids, ammonia, and uric acid. In the feces, nitrogen may be found as amino acids and ammonia. The calculation of nitrogen balance/status is shown in this formula, but stated simply, nitrogen losses are summed and then subtracted from nitrogen intake (In). Thus, nitrogen balance/status = In - [(U - U_e) + (F - F_e) + S].

The subscript e (in U_e and F_e) in the equation stands for *endogenous* (also called *obligatory*) and refers to losses of nitrogen that occur when the subject is on a nitrogen-free diet.

In clinical settings, nitrogen losses are often estimated. Fecal and insensible (including skin, nail, hair) losses of nitrogen are thought to account for about 1 g nitrogen each for a total of 2 g. Urinary losses of nitrogen are measured either as total urinary nitrogen (UN) which gives the most accurate value or as urinary urea nitrogen (UUN). If urinary urea nitrogen is measured, another 2 g nitrogen is usually added to the urea nitrogen losses total to account for the losses in the urine of other nitrogenous compounds such as creatinine, uric acid, ammonia, and so on. Thus, nitrogen balance/status = [protein intake (g)/6.25] - [UN + 2 g] whereby the 2 g accounts for the fecal and insensible nitrogen losses, or nitrogen balance/status = [protein intake (g)/6.25] - [UUN + 2 g + 2 g] whereby the first 2 g accounts for the losses in the urine of nonurea nitrogen compounds and the other 2 g accounts for the fecal and insensible nitrogen losses.

Nitrogen balance studies have been criticized for overestimating true nitrogen retention rates in the body because of incomplete collection or measurement of losses. In addition, nitrogen balance does not necessarily mean amino acid balance; that is, a person may be in nitrogen balance but in amino acid imbalance. The method often used in clinical settings to estimate losses can be quite inaccurate if the individual has larger than normal fecal nitrogen losses (as with diarrhea) or insensible nitrogen losses (as with excessive losses from skin with burns or fever).

Chemical or Amino Acid Score

The chemical score (also called the amino acid score) involves determination of the amino acid composition of a test protein. This procedure is done in a chemical laboratory using either an amino acid analyzer or high performance liquid chromatography techniques. Only the indispensable amino acid content of the test protein is determined. The value is then compared with that of the reference protein, for example, the amino acid pattern of egg protein (considered to have a score of 100). The amino acid/chemical score of a food protein can be calculated as follows:

Table 6.14 Amino Acid Scoring/Reference Patterns and Whole-Egg Pattern [87]

Amino Acid	Children and Adults		
	Infants (mg/g protein)	Adults (mg/g protein)	Whole Egg (mg/g protein)
Histidine	23	18	22
Isoleucine	57	25	54
Leucine	101	55	86
Lysine	69	51	70
Methionine + cysteine	38	25	57
Phenylalanine + tyrosine	87	47	93
Threonine	47	27	47
Tryptophan	18	7	17
Valine	56	32	66

$$\text{Score of test protein} = \frac{\text{Indispensable amino acid in food protein (mg/g protein)}}{\text{Content of same amino acid in reference protein (mg/g protein)}}$$

The amino acid with the lowest score on a percentage basis in relation to the reference protein (egg) becomes the first limiting amino acid, the one with the next lowest score is the second limiting amino acid, and so on. For example, if after testing all amino acids, lysine was found to be present in the lowest amount relative to the reference protein (e.g., 85%), the test protein's chemical score would be 85. The amino acid present in the lowest amount is the limiting amino acid and determines the amino acid or chemical score for the protein. Table 6.14 gives the amino acid pattern in whole egg. Comparison of the quality of different food proteins against the standard of whole-egg protein can be valuable but probably is not nearly as important to adequate protein nutriture as comparison with reference patterns for the various population groups.

Protein Digestibility Corrected Amino Acid Score

The protein digestibility corrected amino acid score (PDCAAS) is another measure of protein quality. This method involves dividing the amount (mg) of the limiting amino acid for a test protein by the amount (mg) of the same amino acid in 1 g of a reference protein (usually egg or milk) and then multiplying this value by the digestibility of the test protein, as shown in the formula below.

$$\text{PDCAAS (\%)} = \frac{\text{Amount (mg) of limiting amino acid in 1 g test protein}}{\text{Amount (mg) of same amino acid in 1 g reference protein}} \times \frac{\text{True Digestibility (\%)}}{\text{Digestibility (\%)}}$$

Examples of foods with a PDCAAS of 100 include milk protein (casein), egg white, ground beef, and tuna, along with some other animal products. Soybean protein has a PDCAAS of 94, and the values for various lentils, peas, and legumes range from <50 to about 70.

Another approach involves a comparison of the amino acid composition of a test protein with a reference *pattern*. The reference pattern that has been selected for use for all people (except infants) is the amino acid requirements of preschool children age 1 to 3 years. The requirements of preschool children, which include needs for growth and development, are higher for each amino acid than are those of adults (who are not undergoing growth and development) and thus should meet or exceed the needs of older people. This overall approach permits evaluation of the protein's ability to meet the nitrogen and indispensable amino acid requirements of people [63].

The scoring pattern, expressed as (mg amino acid)/(g protein), is calculated by dividing the requirements of individual indispensable amino acids (in milligrams) for children by the protein requirements (in grams). The scoring pattern for foods intended for children age 1 year or older and for older age groups is shown in Table 6.14, along with the recommended amino acid scoring pattern for infant formulas and foods, which is based on the amino acid composition of human milk [63]. Table 6.14 also shows the whole-egg pattern.

Protein Efficiency Ratio

The protein efficiency ratio (PER) represents body weight gained on a test protein divided by the grams of protein consumed. To calculate the PER of proteins, young growing animals are typically placed on a standard diet with about 10% (by weight) of the diet as test protein. Weight gain is measured for a specific time period and compared to the amount of the protein consumed. The PER for the protein is then calculated using the following formula:

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Grams of protein consumed}}$$

To illustrate, the PER for casein (a protein found in milk) is 2.5; thus, rats gain 2.5 g of weight for every 1 g of casein consumed. However, a food with a PER of 5 does not have double the protein quality of casein, with a PER of 2.5. Furthermore, although the PER allows determination of which proteins promote weight gain (per gram of protein ingested) in growing animals, no distinction is made regarding the composition (fat or muscle/organ) of the weight gain.

In addition to chemical/amino acid score, protein digestibility corrected amino acid score, and protein efficiency ratio, two additional methods—biological value and net protein utilization—are used to determine protein quality. A discussion of these two methods follows.

Biological Value

The biological value (BV) of proteins is another method used to assess protein quality. BV is a measure of how much nitrogen is retained in the body for maintenance and growth rather than absorbed. BV is most often determined in experimental animals, but it can be determined in humans. Subjects are fed a nitrogen-free diet for a period of about 7 to 10 days, then fed a diet containing the test protein in an amount equal to their protein requirement for a similar time period. Nitrogen that is excreted in the feces and in the urine during the period when subjects consumed the nitrogen-free diet is analyzed and compared to amounts excreted when the subjects consumed the test protein. In other words, the change in urinary and fecal nitrogen excretion between the two diets is calculated. The BV of the test protein is determined through the use of the following equation:

$$\begin{aligned}\text{BV of test protein} &= \frac{I - (F - F_0) - (U - U_0)}{I - (F - F_0)} \times 100 \\ &= \frac{\text{Nitrogen retained}}{\text{Nitrogen absorbed}} \times 100\end{aligned}$$

where I is intake of nitrogen, F is fecal nitrogen while subjects are consuming a test protein, F_0 is endogenous fecal nitrogen when subjects are maintained on a nitrogen-free diet, U is urinary nitrogen while subjects are consuming a test protein, and U_0 is endogenous urinary nitrogen when subjects are maintained on a nitrogen-free diet.

Foods with a high BV are those that provide the amino acids in amounts consistent with body amino acid needs. The body retains much of the absorbed nitrogen if the protein is of high BV. Eggs, for example, have a BV of 100, meaning that 100% of the nitrogen absorbed from egg protein is retained. Although the BV provides useful information, the equation fails to account for losses of nitrogen through insensible routes such as the hair and nails. This criticism is true of any method involving nitrogen balance studies. A further consideration is that proteins exhibit a higher BV when fed at levels below the amount necessary for nitrogen equilibrium, and retention decreases as protein intake approaches or exceeds adequacy.

Net Protein Utilization

Another measure of protein quality involving nitrogen balance studies is net protein utilization (NPU). NPU measures retention of food nitrogen consumed rather than retention of food nitrogen absorbed. NPU is calculated from the following equation:

$$\begin{aligned}\text{NPU of test protein} &= \frac{I - (F - F_0) - (U - U_0)}{I} \times 100 \\ &= \frac{\text{Nitrogen retained}}{\text{Nitrogen consumed}} \times 100\end{aligned}$$

where I is intake of nitrogen, F is fecal nitrogen while subjects are consuming a test protein, F_0 is endogenous fecal nitrogen when subjects are maintained on a nitrogen-free diet, U is urinary nitrogen while subjects are consuming a test protein, and U_0 is endogenous urinary nitrogen when subjects are maintained on a nitrogen-free diet.

Although NPU can be measured in humans through nitrogen balance studies in which two groups of well-matched experimental subjects are used, a more nearly accurate measurement is made on experimental animals through direct analysis of the animal carcasses. In either case, one experimental group is fed the test protein, while the other group receives an isocaloric, protein-free diet. When experimental animals are used as subjects, their carcasses can be analyzed for nitrogen directly (total carcass nitrogen, or TCN) or indirectly at the end of the feeding period. The indirect measurement of nitrogen is made by water analysis. Given the amount of water removed from the carcasses, an approximate nitrogen content can be calculated. NPU involving animal studies is calculated from the following equation:

$$\text{NPU} = \frac{\text{TCN on test protein} - \text{TCN on protein-free diet}}{\text{Nitrogen consumed}}$$

Proteins of higher quality typically cause a greater retention of nitrogen in the carcass than poor-quality proteins and thus have a higher NPU.

Net Dietary Protein Calories Percentage

The net dietary protein calories percentage (NDpCal%) can be helpful in the evaluation of human diets in which the protein:calorie ratio varies greatly. The formula is as follows:

$$\text{NDpCal\%} = \text{Protein kcal}/\text{Total kcal intake} \times 100 \times \text{NPU}_{\text{op}}$$

where NPU_{op} is NPU when protein is fed above the minimum requirement for nitrogen equilibrium.

PROTEIN INFORMATION ON FOOD LABELS

Food labels are required to indicate the amount (quantity) of protein in a food in grams. The % Daily Value for protein also must be specified if the food is intended for consumption by children under 4 years of age or if a health claim is made. To calculate the % Daily Value, the protein quality of the food must be assessed using the protein digestibility corrected amino acid score method if the food is intended for people older than 1 year of age or using the protein efficiency ratio if the food is an infant formula or a baby food.

The Food and Drug Administration specifies the use of the milk protein casein as a standard for comparison of protein quality based on the protein efficiency ratio (PER). Specifically, for infant formulas and baby foods, if a test protein has a protein quality equal to or better than

that of casein—that is, if the PER is ≥ 2.5 —then 45 g of protein is considered to meet 100% Daily Value. If a test protein is lower in quality than casein—that is, if the PER is < 2.5 —then 65 g of protein is needed to meet 100% Daily Value. To establish the protein quality of foods (except foods for infants) for % Daily Value on food labels, a protein digestibility corrected amino acid score (PDCAAS) is used. Specifically, 50 g protein is considered sufficient if the food protein has a PDCAAS equal to or higher than that of milk protein (casein). However, 65 g protein is needed if the protein is of lower quality than milk protein.

RECOMMENDED PROTEIN AND AMINO ACID INTAKES

Protein and amino acid requirements of humans are influenced by age, body size, and physiological state, as well as by the level of energy intake. Multiple studies using multiple methods, especially nitrogen balance studies and the factorial method, have been used to determine protein and amino acid needs of adults. Recent meta-analyses of nitrogen balance studies were considered in the latest recommendations for protein needs for adults. The estimated average requirement for protein for adults (men and women age 19 years and older) was 0.66 g protein per kg body weight or 105 mg nitrogen per kg body weight per day [87]. This value represents the lowest continuing dietary protein intake necessary to achieve nitrogen equilibrium or a zero nitrogen balance in a healthy adult [87]. The recommended dietary allowance (RDA) for protein for adults is 0.8 g protein per kg body weight per day [87]. This RDA value is identical to the 1989 RDA value for protein for adults.

The protein RDAs for children, adolescents, and adults, including women during pregnancy and lactation, are provided on the inside cover of this book. Instead of RDA, the recommendations for protein for infants from birth to 6 months of age are given as an adequate intake (AI). The AI was derived based on data from infants fed human milk as the primary nutrient source for the first 6 months [87].

In addition to recommendations for protein intake, RDAs for the indispensable amino acids have also been established. These recommendations, based on a variety of methods including amino acid balance and oxidation studies and indicators of amino acid oxidation, are shown in Figure 6.45. The reader is directed to the Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Protein and Amino Acids [87] for in-depth information on the methods used in determining the recommendations for the amino acids and protein.

No tolerable upper intake level for protein or any of the amino acids has been established. An upper level of recommended protein intake based on percentage of total energy (kcal) in the diet supplied by protein has been established at 30% [87]. Long-term effects from ingestion of a diet supplying $>30\%$ of kilocalories from protein

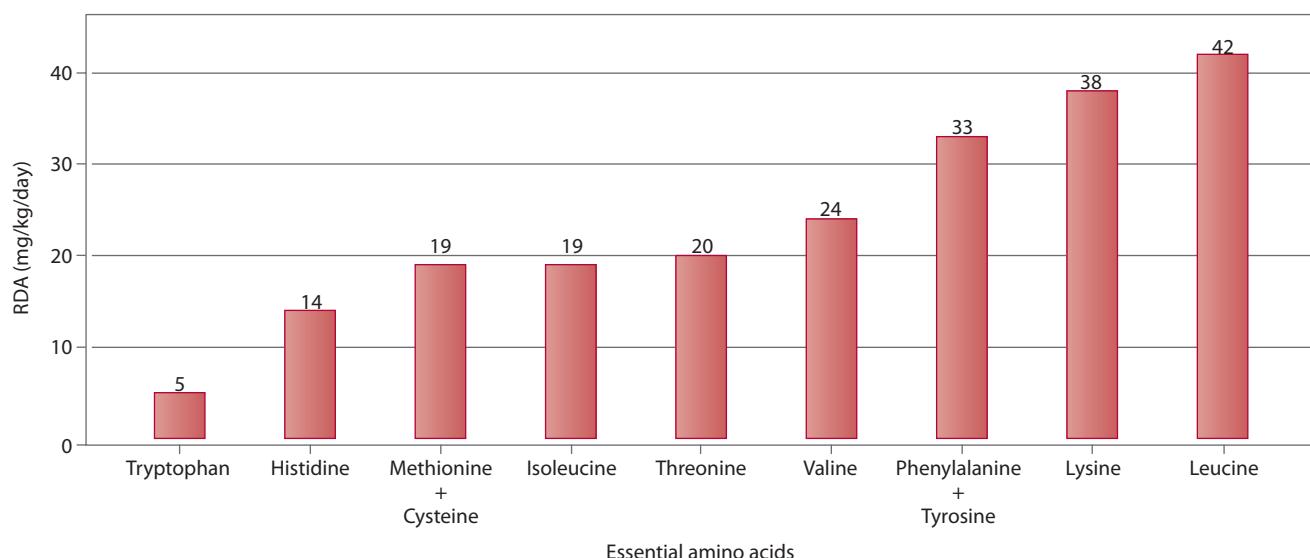


Figure 6.45 Recommended dietary allowances for indispensable amino acids for adults.

Source: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Food and Nutrition Board, Institute of Medicine, Washington DC, National Academic Press, 2005, p680.

have not been investigated. Although scientific data are sparse from studies or reports of people consuming relatively large amounts of dietary protein, a few population groups routinely ingest high protein diets. Weight lifters and body builders, for example, often ingest up to 3 g protein/kg body weight, higher than the recommendation of about 1.2 to 1.8 (or up to 2) g protein/kg body weight for athletes. Thus, intakes of 300 g protein per day are not uncommon among some athletes [88].

Whether high protein diets are detrimental to health is controversial [89–102]. The most commonly cited hazards include increased risk of dehydration, and possible kidney damage and bone damage. Dehydration, caused by the need to excrete large amounts of urea and other nitrogenous wastes from protein catabolism, can be prevented with appropriate fluid consumption. Renal damage in people with no prior history of renal problems has not been widely reported. Effects on bone vary; some studies suggest catabolic effects, and others suggest anabolic effects. The catabolic effects of a high protein diet on bone are most commonly attributed to the generation of large amounts of acids from the dietary protein, although other mechanisms have been proposed, such as increased osteoclast activity associated with decreased nitric oxide synthesis (the high protein diet upregulates the urea cycle with less arginine available for nitric oxide synthesis, which inhibits osteoclast activity) [102].

The effects of an acid ash load are briefly reviewed here. Acid as well as alkaline ash is produced in the body in varying amounts, based on the foods consumed. For example, eating milk, yogurt, and fruits and vegetables produces more alkaline ash than acid ash. In contrast, ingestion of meat, fish, eggs, cheese and, to a lesser extent, most grain

products generates more acid ash. Most of the acids generated by these protein-rich foods is thought to arise from oxidation of the sulfur-containing amino acids. Soft drinks (consumed in large quantities), however, also provide considerable amounts of acids (especially phosphoric acid) that are absorbed into the body. Excess acids in the body are excreted in the urine; however, the pH of the urine can only go so low—usually not less than 5. A low-grade metabolic acidosis (in the blood) is thought to be generated by the ingestion of large amounts of high protein foods (and soft drinks) and inadequate amounts of fruits and vegetables that supply bicarbonate and other substances that can help to buffer the acids. If the kidneys cannot excrete the excess acid load, and in the absence of adequate fruit and vegetable intakes to supply buffers, the buffering is thought to occur at the expense of bone, which releases calcium, magnesium, and carbonate, among other compounds, to serve as the buffers. (See also the Chapter 11 Perspective “Osteoporosis and Diet.”)

Alternately, high protein diets have been shown to promote anabolic effects on bone and even reduce fracture risk in older people. Moreover, some studies have suggested that elderly women may need more protein (>0.84 g protein/kg body weight) than is currently recommended to optimize bone mass [89,90]. Various mechanisms for the anabolic effects of protein on bone have been proposed. Amino acids, for example, are needed for the synthesis of proteins in bones. Moreover, amino acids and diets high in protein stimulate insulin-like growth hormone I, which promotes bone growth. It has also been found that in most individuals, protein intake is associated with increased phosphorus and calcium intakes, which diminish calcium losses. Further, any decreases in serum calcium

concentrations caused by increased protein-induced calcium losses would stimulate parathyroid hormone secretion, increase active vitamin D synthesis, and thus increase calcium absorption. Effects on the bone are then thought to depend (at least partially) on the amount of calcium in the diet and the body's ability to make these hormonal changes [95]. Clearly, the relationship between protein and bone health requires additional study, as does the long-term effects of a high protein diet on health.

To help guide decisions in choosing good sources of protein, the United States Department of Agriculture (USDA) published the Food Guide and MyPyramid, which include six major food groups. MyPyramid is designed for the consumer and can be accessed at www.mypyramid.gov. From this site, an individual can determine the appropriate amount of foods recommended from each of the food groups. The amounts vary based on a person's gender, age, and level of physical activity. Generally, however, the recommended servings from the meat, poultry, fish, eggs, beans, nuts and seed group range from 2 to 7 oz per day and the recommended servings from the dairy group range from 2 to 3 cups per day depending on gender, age, and level of physical activity. Foods ingested from these protein-rich food groups should also be low in fat. Grains also provide some protein. Choices from this group should be high in fiber and low in fat; recommended servings from the grain group range from 3 to 10 cups or the equivalent per day depending on gender, age, and level of physical activity.

In addition to the RDA for protein, the Institute of Medicine has published an Acceptable Macronutrient Distribution Range for protein of 10% to 35% of energy intake. Use of this range is appropriate as long as the intake of energy is adequate. If, for example, a person only ingests 800 kcal per day, then 10% to 35% of energy equals 80 to 280 kcal, which at 4 kcal/g protein is equal 20 to 70 g protein. An intake of 20 g protein is not sufficient for an adult

to maintain nitrogen balance; however, depending on the age, gender, and body weight of the individual, 70 g may be more than adequate.

PROTEIN DEFICIENCY/MALNUTRITION

Protein deficiency can occur in those ingesting inadequate protein with or without adequate energy (kcal). Kwashiorkor is one form of protein malnutrition whereby people typically receive enough energy usually as carbohydrate, but insufficient protein intakes. Kwashiorkor is characterized by poor (inadequate) visceral protein status which is manifested by below normal concentrations of total protein, albumin, retinol-binding protein, and prealbumin (transthyretin) in the blood. Without enough of these visceral proteins in the blood, water diffuses out of the blood (the intravascular space) into interstitial (intercellular) spaces causing edema (swelling). The edema usually appears first in the legs, but may be also present in the face or more generalized over the body. Body weight, muscle mass, and adipose mass may be normal in those with kwashiorkor. The condition is widely seen in developing countries, but is also seen in persons who are hospitalized with conditions such as burns, sepsis, trauma, or following surgery. In these situations, protein needs are exceptionally high and if the patient fails to consume adequate protein, malnutrition ensues.

Marasmus is a second form of protein malnutrition. People with marasmus are typically extremely thin (emaciated or underweight) with wasted (depleted) muscle mass and adipose tissue. Bones are prominent in appearance and the skins often droops or hangs from the body. Indicators of visceral protein status are typically within the normal range or just below the normal range, but not to the extent seen in kwashiorkor. Marasmus typically results from a chronic (prolonged) period of insufficient energy and protein intakes.

SUMMARY

Proteins in foods become available for use by the body after they have been broken down into their component amino acids. Nine of these amino acids are considered essential; therefore, the quality of dietary proteins correlates with their content of these indispensable amino acids. In the body, proteins play many vital roles including functions in structural capacities, and as enzymes, hormones, transporters, and immunological protectors, among other roles.

An important concept in protein metabolism is that of amino acid pools, which contain amino acids of dietary origin plus those contributed by the breakdown of body tissue. The amino acids comprising the pools are used in a variety of ways: (1) for synthesis of new proteins for growth and/or replacement of existing body proteins; (2)

for production of important nonprotein nitrogen-containing molecules; (3) for oxidation as a source of energy; and (4) for synthesis of glucose, ketones, or fatty acids.

The liver is the primary site of amino acid metabolism, but no clear picture of the body's overall handling of nitrogen can emerge without considering amino acid metabolism in a variety of tissues and organs. Of particular significance is the metabolism of the branched-chain amino acids in the skeletal muscle and the production of the ammonium ion in the kidney. In addition, current research on neuropeptides spotlights the importance of amino acid metabolism in neural tissue.

Of the nonessential amino acids, glutamine, glutamate, and alanine assume particular importance because of the versatility in the overall metabolism of these amino acids.

Glutamate and its α -keto acid make possible many crucial reactions in various metabolic pathways for amino acids. An appreciation for the functions performed by glutamine and glutamate makes one realize that the term “dispensable” as applied to these amino acids may be misleading.

Protein metabolism is particularly responsive to hormonal action, and this action can vary according to the tissue effect. Protein metabolism as regulated by hormonal action is particularly significant during periods of stress (see the Perspective).

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Suggested Readings

Proceedings of the 5th Amino Acids Assessment Workshop. *J Nutr* 2006; 136(6 Suppl):1633S–1757S.

Protein Turnover: Starvation Compared with Stress

Starve a cold, feed a fever? Or should we feed a cold and starve a fever? In the healthy adult, protein synthesis approximately balances protein degradation. Together, protein synthesis and degradation make up protein turnover. Protein turnover in humans is correlated to a person's mass ($W^{0.75}$), where W is body weight in kilograms. Daily protein turnover in humans is calculated to be approximately 4.6 g/kg body weight. Thus, for a 70 kg male, protein turnover approximates 320 g daily [1]. (Remember, 1 pound equals 454 g.) However, such calculations only approximate reality in a healthy adult. With illness, such as infection or sepsis (the presence of pathogenic microorganisms or their toxins in the blood and/or body tissues), or during starvation, protein synthesis and protein degradation are not in balance. An imbalance between protein synthesis and protein degradation is also found with injury, including surgery, trauma, and burns; however, this imbalance exceeds that found in fasting (starvation) conditions. Conditions of illness or injury comprise what is referred to as "stress" in the title of this Perspective. This Perspective compares what happens to a person's protein status during starvation with the effects of illness or injury (i.e., stress).

In starvation or when food is not consumed for prolonged periods, protein synthesis decreases. This decrease occurs because of a reduction in mRNA needed for the translation of proteins and because of a decreased rate of peptide bond formation (or RNA "activity"). Even proteins with very rapid turnover, such as plasma proteins, are synthesized at a rate 30% to 40% below normal. In muscle, protein synthesis rates drop even lower. However, protein degradation rates decrease concurrently so that in chronic starvation daily losses of nitrogen become quite small, about 4 to 5 g urinary nitrogen per day; however, differences in the extent of nutrient metabolism between lean and obese people have been reported [2,3].

The principal mechanism of adjustment to starvation is a change in hormone balance. In particular, insulin production decreases sharply. In addition, the muscle and adipocytes become somewhat resistant to the action of insulin, so that whatever insulin is circulating is ineffective in promoting cellular nutrient uptake for protein synthesis and lipogenesis. Decreased insulin activity, coupled with increased synthesis of counterregulatory hormones such as glucagon, promotes fatty acid mobilization from adipose tissue, production of ketones, and the availability of amino acids for gluconeogenesis. The glucocorticoids, especially cortisol, are important in gluconeogenesis because

they promote catabolism of muscle protein to provide substrates for gluconeogenesis. However, an increased adjustment to starvation is characterized by a decrease in the secretion of glucocorticoids. An additional hormonal change facilitating adjustment to starvation is decreased synthesis of tri-iodothyronine (T_3 , a thyroid hormone), which results in a lowered metabolic rate.

In the initial stages or first few days of fasting or starvation, glycogen in the liver is depleted. Muscles undergo proteolysis. Urinary 3-methylhistidine excretion increases to reflect myofibrillar protein catabolism. Muscles undergoing proteolysis also release into the blood a mixture of amino acids containing relatively high alanine and glutamine concentrations. Alanine is a preferred substrate for gluconeogenesis and serves to stimulate the secretion of the gluconeogenic hormone glucagon. Alanine released from muscle is taken up by the liver, where the nitrogen is removed and converted to urea for excretion by the kidney and where the pyruvate can be used to make glucose by way of the gluconeogenic pathway. Glucose is also made in the liver from recycled lactate and pyruvate (the Cori cycle) and from glycerol released from adipose tissue lipolysis [2]. Glucose formed in the liver may be released into the blood for cellular uptake and metabolism. Glutamine released from muscles circulates in the blood for uptake and metabolism primarily by the gastrointestinal tract and over time especially by the kidney.

As fasting or starvation continues, tissues continue to use fatty acids and glucose for energy but also begin to use ketones formed in the liver from fatty acid oxidation. A decrease in protein catabolism (and thus urea synthesis) and gluconeogenesis occurs concurrently with the brain's and other tissues' adaptation to ketones as a source of energy. Acidosis increases, however, as ketone production accelerates. Consequently, more glutamine is directed to the kidneys (and away from other organs) for maintenance of acid-base balance. In the kidney, the amino groups from the glutamine are used to produce ammonia. Ammonia can combine with hydrogen ions and be excreted in the urine to help correct the acidosis. The carbon skeleton from the glutamine is used in the kidney to make glucose by gluconeogenesis. After about 5 to 6 weeks fasting, splanchnic glucose production totals about 80 g per day with 10 to 11 g glucose per day synthesized from ketones, 35 to 40 g glucose per day made from recycled lactate and pyruvate, 20 g per day from glycerol, and 15 to 20 g glucose made from amino acids (mostly alanine) released from muscle [2].

Figure 1a illustrates how adaptation to starvation allows the conservation of body protein. Fatty acids are

shown generating ketones that are then used for energy by muscle. The use of the ketones means that less glucose is needed and allows the sparing of lean body mass for glucose production. In other words, because less carbohydrate (glucose) is required by the body, less protein must be broken down to supply amino acids for gluconeogenesis. Amino acids resulting from proteolysis of muscle tissue can be used for the synthesis of crucial visceral proteins such as plasma proteins, which have more rapid turnover rates than muscle. It is estimated that under normal conditions visceral protein has a turnover rate three times greater than that of muscle protein [4].

Figure 1b depicts the substrate use in sepsis. Patients with sepsis are hypermetabolic; that is, their basal metabolic rate is elevated above normal because of the infection. People with burns and trauma (including surgery) also have elevated metabolic rates. The length and severity of hypermetabolism varies with the severity of the stress. For example, with minor surgery or injury, the rise in metabolism (and the catabolic state that also occurs) may last less than a week, whereas with severe burns and multiple trauma, it may last several months. With sepsis (an example of stress), as with starvation, catabolism of body tissues occur. Adipose tissue undergoes lipolysis. However, in stress, the fatty acids generated from lipolysis do not produce ketones. Thus, with stress the body cannot defend itself and diminish muscle catabolism. Ketogenesis is inhibited by insulin during sepsis, burns, injury or trauma, and surgery. Without the use of ketones, body protein must continue to be degraded to supply amino acids for glucose synthesis (gluconeogenesis). Degradation of muscle protein provides most of the amino acids needed by other organs. In stress situations, muscles experience decreased amino acid uptake and protein synthesis with larger increases in protein breakdown. Degradation of white fast-twitch skeletal muscle is more pronounced than that of red slow-twitch skeletal muscle [5]. Urinary 3-methylhistidine excretion increases, reflecting increased catabolism of myofibrillar protein. Muscle cachexia, characterized by muscle wasting and weakness, results [6]. Thus, protein degradation in sepsis, as well as in injury, trauma, and burns, exceeds that in starvation. Each gram of nitrogen lost can be translated into the breakdown of approximately 30 g of hydrated lean tissue [7]. (This 30 g figure is based on the following: 1 g nitrogen = 6.25 g protein and muscle is about 80% water. So, 30 g muscle = 24 g water + 6.25 g protein. The 24 g water is 80% of the 30 g.)

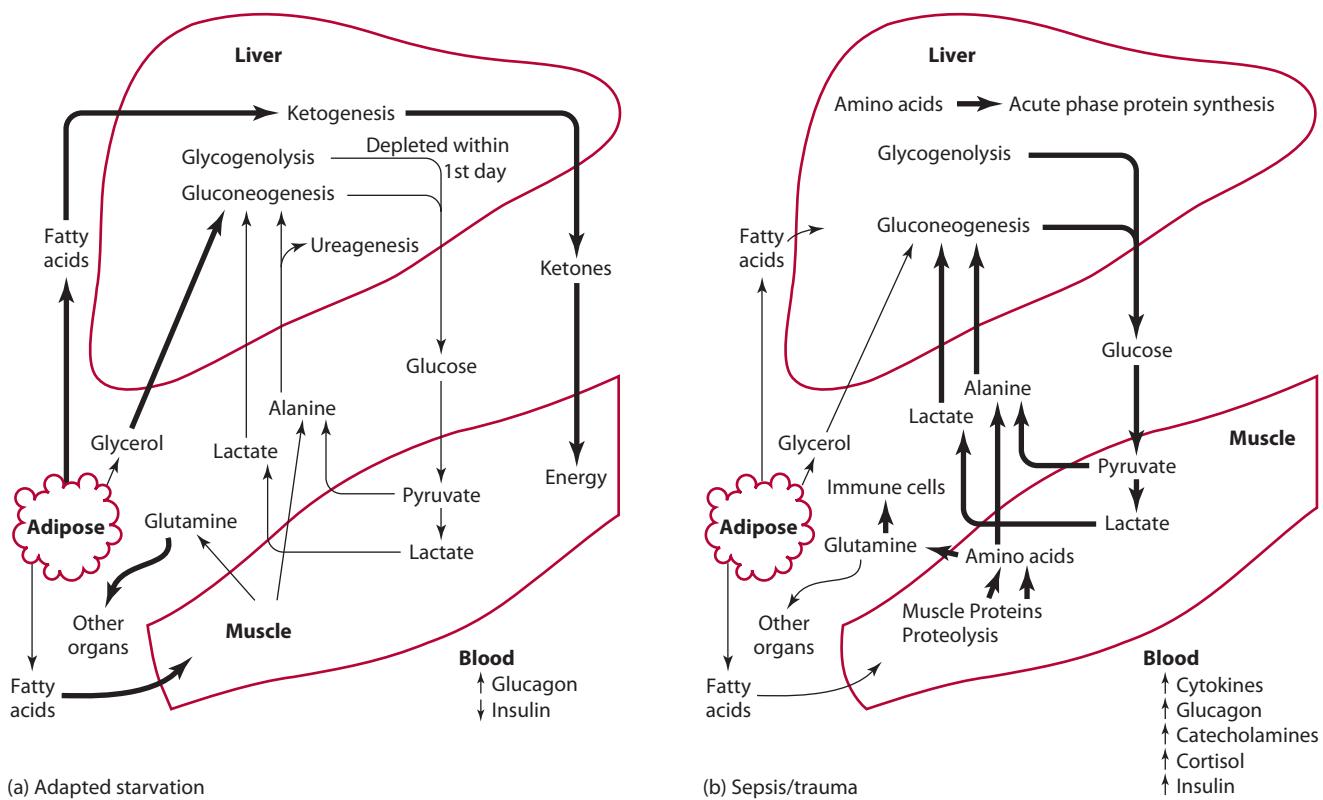


Figure 1 Substrate utilization during (a) adapted starvation and (b) sepsis/trauma. Note: Increased responses are shown by heavy black arrows.

The differences in substrate use between starvation and stress result in part from differences in hormone concentrations. Figure 2 demonstrates the metabolic stress response. As shown in this diagram, with stress (including sepsis, trauma, surgery, and burns), glucocorticoids (primarily cortisol), catecholamines (e.g., epinephrine), insulin, and glucagon release increase. However, despite the presence of insulin, the body's tissues become resistant to insulin action, and hyperglycemia (high blood glucose concentration) persists. In addition, cortisol concentrations may remain elevated in the blood for prolonged periods following severe trauma or stress events. High blood cortisol promotes proteolysis and hyperglycemia. Besides hormone release, cytokines contribute to differences in substrate use during stress. Cytokines are low-molecular-weight peptides that evoke a number of varied reactions in the body and are used primarily by immune cells to communicate with each other. Cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF)- α produced from macrophages in part mediate proteolysis and the hormonal response.

Inflammation involves similar cytokines, such as IL-1 and TNF- α , but also interleukin (IL)-6, interleukin (IL)-8, and interferon (IFN)- γ .

Additional hormonal changes associated with stress, illustrated in Figure 2, include the release of aldosterone and antidiuretic hormone. Aldosterone promotes renal sodium and fluid reabsorption, thus increasing blood volume. Antidiuretic hormone (ADH) inhibits diuresis (urination), also effecting an increase in blood volume. Both aldosterone and ADH help restore circulation if it has been depressed by shock or loss of blood fluids by hemorrhage associated with injury or surgery. These hormones thus help diminish total fluid losses, which may be high with skin loss from burns or with increased dermal losses from fever.

Although surgery, sepsis, burns, and trauma are associated with continued protein degradation to supply amino acids for glucose synthesis, protein turnover also occurs because of the immune response and the acute phase response. The acute phase response is characterized by fever, hormonal changes, and blood

cell count changes, as well as by changes in protein turnover. During the acute phase response, certain body proteins such as muscle protein are preferentially degraded by ubiquitin/proteasomal and calpain/calcium systems; however, in the liver, protein synthesis predominates. Glucocorticoids and cytokines, in part, are thought to stimulate the increase in the synthesis of some proteins in the liver. Proteins that are synthesized in the liver during these stress situations include primarily a group of proteins called acute phase reactant (APR) proteins or acute phase response proteins (APRPs). Some of these proteins also appear to be synthesized and/or degraded by macrophages, lymphocytes, and fibroblasts; thus, these cells help modulate the body's response [8]. In contrast, the synthesis of some proteins, such as the plasma proteins albumin and transferrin, diminishes with stress situations. Some examples of APR proteins and their functions include:

- haptoglobin, a protein that binds free hemoglobin (hemoglobin not in the red blood cell) that has been released by hemolysis of the red blood cell

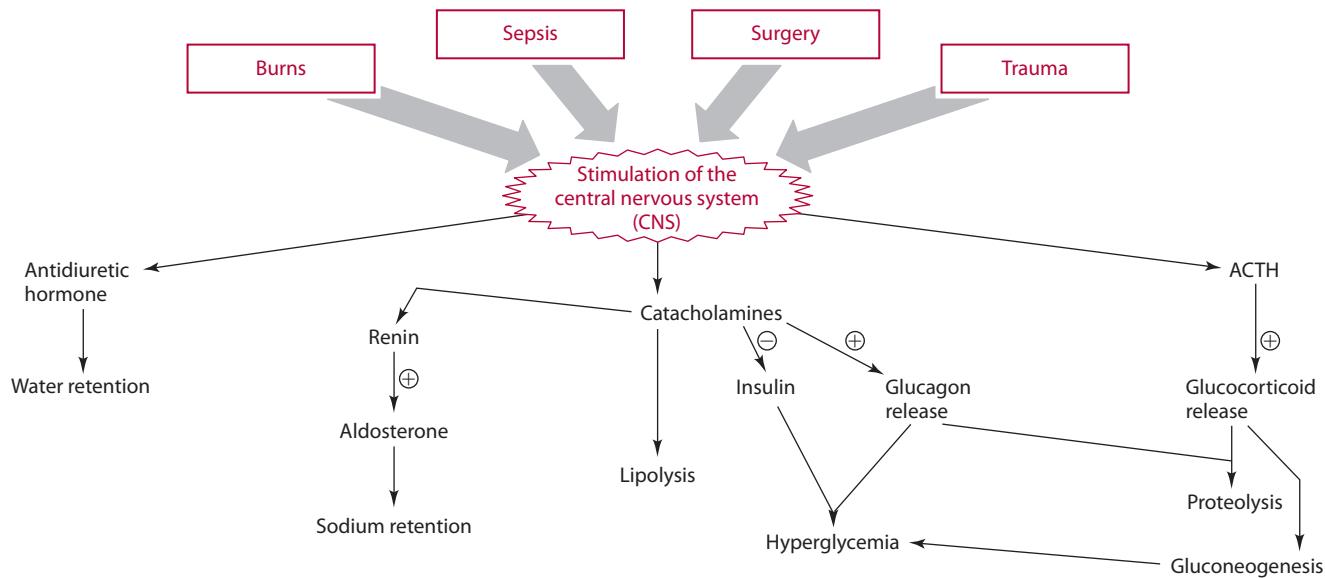


Figure 2 Response to metabolic stress.

- ceruloplasmin, a copper-containing protein with oxidase activity and the ability to scavenge radicals
- α 1 macroglobulin, a protease inhibitor that functions to effect changes in tissue damage and restructuring
- α 1 antitrypsin, a protease inhibitor that minimizes further tissue damage associated with phagocytosis of microorganisms
- fibrinogen, a protein required for blood coagulation
- C-reactive protein, a protein that stimulates phagocytosis by white blood cells and activates complement proteins, which are needed for antibody-induced destruction of microorganisms
- orosomucoid (α 1 acid glycoprotein), a protein necessary for wound healing
- serum amyloid A, a protein involved in the oxidative burst

In addition to the synthesis of these proteins, more metallothionein (a zinc-containing protein) is made in the liver with sepsis and inflammation. Consequently, hepatic zinc concentrations increase while plasma zinc concentrations decrease. Similarly, in sepsis, the concentrations of iron and iron-containing proteins in the blood diminish while hepatic iron stores as ferritin rise.

However, when whole-body protein turnover is considered, the increased rate of hepatic synthesis of protein with sepsis and other stress situations is insignificant compared to the rate of protein degradation.

Whole-body protein catabolism predominates over synthesis. Moreover, inadequate dietary protein intake can diminish the magnitude and pattern of the acute phase response as well as the ability of the body to synthesize antioxidant defense compounds such as glutathione [9–11]. Even a delay in or the lack of enteral (meaning by way of or into the gastrointestinal tract) nutrition, which may accompany severe illness, can result in the atrophy of intestinal mucosa. When enterocytes atrophy, bacteria or toxins can more easily translocate from the lumen of the intestinal tract through the enterocytes and into the blood. Such bacterial translocation further increases the risk of sepsis, especially gram-negative sepsis. Glutamine has been shown to be a vital fuel for enterocytes. Moreover, the glutamine needs of the cells of the immune system also are increased with sepsis and other stress conditions. Yet, during illness, the rate of glutamine production and release from body sites does not meet the intestinal cells' need for glutamine. Further, lack of sufficient glutamine may contribute to inadequate glutathione production observed in stress [12,13]. Glutathione, remember, is an important antioxidant. A glutamine-enriched diet for patients hospitalized

with such catabolic illnesses has been shown to help maintain the integrity of the intestine, decrease intestinal permeability, help preserve skeletal muscle, decrease infectious complication rates, and decrease the length of hospital stay [14]. Thus, glutamine becomes a conditionally indispensable amino acid during times of sepsis or injury.

It has been suggested [7] that the body places a high priority on wound repair and host defense, gambling that convalescence will occur before depletion of tissues becomes a threat to survival. To improve recovery, feed a cold and feed a fever!

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Integration and Regulation of Metabolism and the Impact of Exercise and Sport

Interrelationship of Carbohydrate, Lipid, and Protein Metabolism

The Central Role of the Liver in Metabolism

Tissue-Specific Metabolism during the Fed-Fast Cycle

Carbohydrate and Lipid Metabolism

Amino Acid Metabolism

System Integration and Homeostasis

Endocrine Function in Fed State

Endocrine Function in Postabsorptive or Fasting State

Metabolic Syndrome

Insulin Resistance

Weight Loss and Insulin Insensitivity

Sports Nutrition

Biochemical Assessment of Physical Exertion

Energy Sources during Exercise

Carbohydrate Supplementation (Supercompensation)

Diets for Exercise

Nutritional Ergogenic Aids

Summary

PERSPECTIVE

Diabetes: Metabolism Out of Control

Chapters 3, 5, and 6 featured carbohydrate, lipid, and protein metabolism at the level of the individual cell, with emphasis on metabolic pathways common to nearly all eukaryotic cells. Those chapters also discussed how the pathways are regulated at the level of certain regulatory enzymes by substrate availability, allosteric mechanisms, and covalent modifications such as phosphorylation.

For their significance to be fully appreciated, metabolic pathways—and the specific metabolic roles of different organs and tissues—must be viewed in the context of the whole organism. Therefore, in this chapter we examine (1) how the major organs and tissues interact through integration of their metabolic pathways, (2) hormonal regulation of these metabolic processes in maintaining homeostasis, (3) examples of the body's ability to maintain homeostasis under special circumstances of fasting and intense exercise, and (4) a discussion of what happens as the control is lost leading to "metabolic syndrome." The pathways themselves are not reproduced again in this chapter. When appropriate, the reader is referred to pertinent sections in previous chapters where the pathways are described. A section on the currently attractive field of sports nutrition is included at the end of this chapter. This topic is presented at this point in the text because the dynamics of substrate use in supplying energy for physical exercise provide a practical example of how the various metabolic pathways interrelate. Skeletal muscle represents 43% of body mass by weight. During strenuous exercise, skeletal muscle uses a disproportionate amount of the body's energy reserves.

The interrelationship among carbohydrates, lipids, and proteins has been alluded to in the previous chapters. Each of these macronutrients is involved in both anabolic and catabolic reactions. Generally, anabolic reactions require energy, and catabolic reactions produce energy. Much of the interrelationship among the macronutrients centers around the flux of energy and the availability of substrates. This interrelationship is discussed in more detail in this chapter.

Interrelationship of Carbohydrate, Lipid, and Protein Metabolism

If ingested in sufficient amounts, any of the three energy-producing nutrients—carbohydrate, fat, and protein (amino acids)—can provide the body with its needed energy on a short-term basis. Within certain limitations, anabolic interconversion among the nutrients also occurs. For example, as explained in Chapter 6, certain amino acids can be synthesized in the body from

carbohydrate or fat, and, conversely, most amino acids can serve as precursors for carbohydrate or fat synthesis. An overview of the considerable metabolic interconversion among the nutrients is given in Figure 7.1. Not evident from the figure, but very important to recall, is that the TCA cycle is an **amphibolic pathway**, meaning that it not only functions in the oxidative catabolism of carbohydrates, fatty acids, and amino acids but also provides precursors for many biosynthetic pathways, particularly gluconeogenesis (Figure 3.17). Along with pyruvate, several TCA cycle intermediates—including α -ketoglutarate, succinate, fumarate, and oxaloacetate—can be formed from the carbon skeletons of certain amino acids and can function as gluconeogenic precursors.

The fact that animals can be fattened on a predominantly carbohydrate diet is evidence of the apparent ease by which carbohydrate can be converted to fat. However, human lipogenesis from glucose is now believed to be

much less efficient than previously proposed [1], and weight gain from carbohydrate is thought to be caused by sparing lipolysis rather than direct carbohydrate lipogenesis [2]. Glucose is the precursor for both the glycerol and the fatty acid components of triacylglycerols. The glycerol portion can be formed from dihydroxyacetone phosphate (DHAP), a three-carbon intermediate in glycolysis (Figure 3.17). Reduction of DHAP by glycerol 3-phosphate dehydrogenase and NADH produces glycerol 3-phosphate, to which CoA-activated fatty acids attach in the course of triacylglycerol synthesis (Figure 5.32). An extremely important reaction, which links glucose metabolism to fatty acid synthesis, is that of the pyruvate dehydrogenase complex. This reaction converts pyruvate to acetyl CoA by dehydrogenation and decarboxylation. Acetyl CoA is the starting material for the synthesis of long-chain fatty acids as well as a variety of other lipids (Figure 5.27 and Figure 7.2).

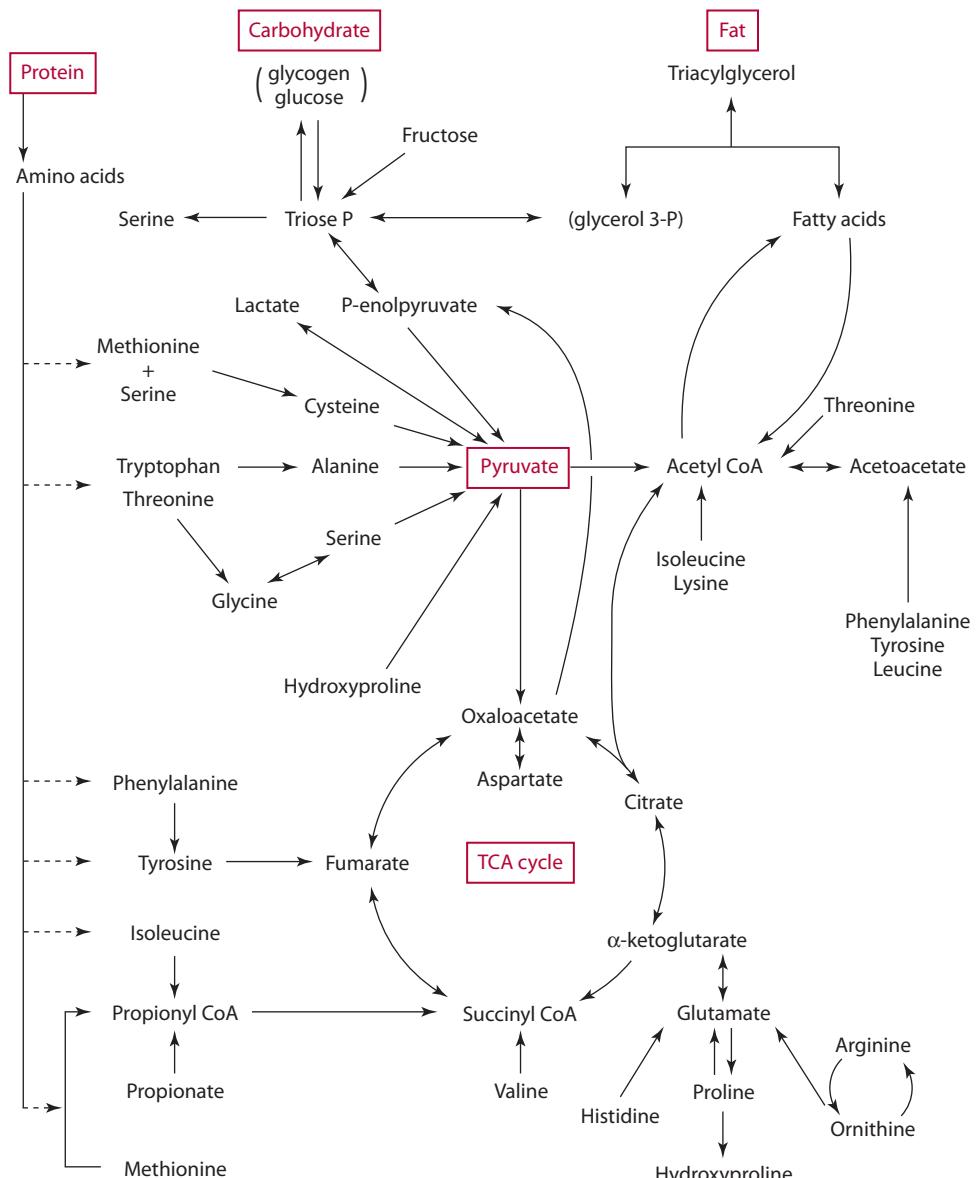


Figure 7.1 Interconversion of the macronutrients.

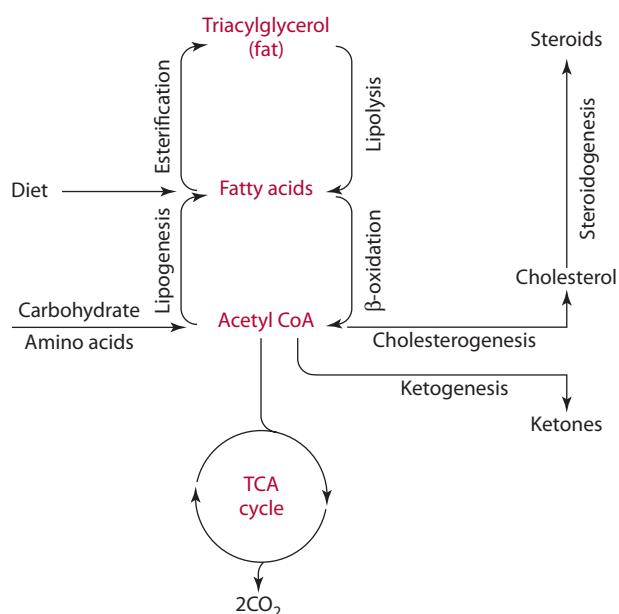


Figure 7.2 Overview of lipid metabolism, emphasizing the central role of acetyl CoA.

Although carbohydrate can be converted into both the glycerol and the fatty acid components of fat, only the glycerol portion of fat can be converted to carbohydrate. The conversion of fatty acids into carbohydrate is not possible because the *pyruvate dehydrogenase reaction is not reversible*. This fact prevents the direct conversion of acetyl CoA, the sole catabolic product of even-numbered-carbon fatty acids, into pyruvate for gluconeogenesis. In addition, gluconeogenesis from acetyl CoA as a TCA cycle intermediate cannot occur, because for every two carbons in the form of acetyl CoA entering the cycle, two carbons are lost by decarboxylation in early reactions of the TCA cycle (Figure 3.19). Therefore, there can be no net conversion of acetyl CoA to pyruvate or to the gluconeogenic intermediates of the cycle. Consequently, acetyl CoA produced from whatever source must be used for energy, lipogenesis, cholesterogenesis, or ketogenesis.

Although fatty acids that have an even number of carbons are degraded exclusively to acetyl CoA and therefore are not glucogenic (gluconeogenic) for the reasons mentioned, fatty acids that possess an odd number of carbon atoms are partially glucogenic. Fatty acids with an odd number of carbons can be partially converted to glucose because propionyl CoA ($\text{CH}_3-\text{CH}_2-\text{COSCoA}$), ultimately formed by β -oxidation, is carboxylated and rearranged to succinyl CoA, a glucogenic TCA cycle intermediate (Figure 5.25). Fatty acids with an odd number of carbon atoms are not common in the diet.

The glycerol portion of all triacylglycerols is glucogenic, entering the glycolytic pathway at the level of DHAP (Figure 3.17). Following its release from triacylglycerol by lipase hydrolysis, glycerol can be phosphorylated to glycerol 3-phosphate by glycerokinase, then oxidized to

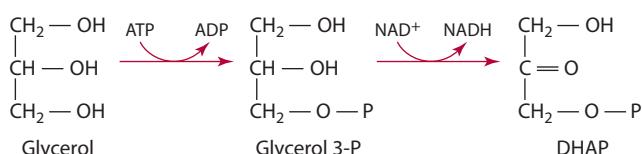


Figure 7.3 Phosphorylation and oxidation of glycerol to dihydroacetone phosphate (DHAP).

DHAP by glycerol 3-phosphate dehydrogenase (Figure 7.3). During the fasting state, when fat catabolism is accelerated, this conversion assumes greater importance in maintaining a normal level of blood glucose.

Metabolism of the amino acids gives rise to a variety of amphibolic intermediates, some of which produce glucose, while others produce the ketone bodies by their conversion to acetyl CoA or acetoacetyl CoA (Figure 7.3). The catabolism of the individual amino acids is covered in Chapter 6. Remember that amino acids that can be used for production of glucose are termed glucogenic, and those producing ketones are called ketogenic. Only the amino acids leucine and lysine are purely ketogenic. The dispensable (nonessential) glucogenic amino acids are usually interconverted with carbohydrate, but like the ketogenic amino acids, they can be converted (indirectly) into fatty acids by first being converted to acetyl CoA which is a precursor for fatty acid synthesis. Fatty acids cannot be converted into the glucogenic amino acids for the same reason that fatty acids cannot form glucose—namely, the irreversibility of the pyruvate dehydrogenase reaction. Although entirely possible, the conversion of the glucogenic amino acids into fat is rather uncommon. Only when protein is supplying a high percentage of calories would glucogenic amino acids be expected to be used in fat synthesis. All the amino acids producing acetyl CoA directly—isoleucine, threonine, phenylalanine, tyrosine,* lysine, and leucine—are indispensable.

The interconversion of the energy-producing nutrients appears to be skewed toward providing the organism with an energy source that can be easily stored (fat), thereby providing for times when food is not readily available. Energy released by the catabolic processes of the major nutrients must be shared by the energy-requiring synthetic pathways discussed earlier. On reaching the cells, the energy-producing nutrients can be catabolized to produce phosphorylative energy (ATP), reductive energy (NADH, NADPH, FADH_2), or both. Alternatively, the energy-producing nutrients may be synthesized into more complex organic compounds or macromolecules that become cellular components. For synthesis of a cellular component to occur, however, chemical energy must be provided. Therefore, when the cell places priority on synthesizing a particular component, another energy-producing material

*Tyrosine is formed by hydroxylation of phenylalanine; therefore, its carbon skeleton cannot be synthesized in the body but must be obtained from food.

must be catabolized. The common energy pool within a cell is finite, and all anabolic and endergonic processes compete for this energy. For example, when the liver is producing more glucose by reversing glycolysis (i.e., gluconeogenesis), it cannot be synthesizing lipids and proteins at the same time. Instead, some of the existing cellular proteins or lipids are hydrolyzed, and the resulting amino acids or fatty acids are oxidized to generate the NADH and ATP needed for gluconeogenesis. Likewise, when hepatic lipogenesis occurs, glucose must be used to produce the NADPH and ATP necessary for the conversion of acetyl CoA to fatty acids.

The final common catabolic pathway for carbohydrate, fat, and protein is the TCA cycle and oxidative phosphorylation as part of the electron transport chain (Figures 3.19 and 3.26). In addition to releasing energy, these mitochondrial processes are crucial for many other metabolic sequences:

- CO₂ produced by oxidation of acetyl CoA is a source of cellular carbon dioxide for carboxylation reactions that initiate fatty acid synthesis and gluconeogenesis. This CO₂ also supplies the carbon of urea and certain portions of the purine and pyrimidine rings (Figures 6.16, 6.20, and 6.26).
- The TCA cycle provides common intermediates that provide the cross-linkages between lipid, carbohydrate, and protein metabolism, as illustrated in Figure 7.1. Particularly notable intermediates are α-ketoglutarate and oxaloacetate. Another interrelationship, not shown in Figure 7.1, is that between heme and an intermediate of the TCA cycle, succinyl CoA. The initial step in heme biosynthesis is the formation of α-aminolevulinic acid from “active” succinate and glycine (Figure 12.6).
- TCA cycle intermediates—citrate and malate—intermesh with lipogenesis. Citrate can move from the mitochondria into the cytoplasm, where citrate lyase cleaves it into oxaloacetate and acetyl CoA, the initiator of fatty acid synthesis. Malate, in the presence of NADP⁺-linked malic enzyme, may provide a portion of the NADPH⁺ required for reductive stages of fatty acid synthesis.

The Central Role of the Liver in Metabolism

Each tissue and organ of the human body has a specific function that is reflected in its anatomy and its metabolic activity. For example, skeletal muscle uses metabolic energy to perform mechanical work, the brain uses energy to pump ions against concentration gradients to transfer electrical impulses, and adipose tissue serves as a depot

for stored fat, which on release provides fuel for the rest of the body. Central to all these processes is the liver. It plays the key role of processor and distributor in metabolism, furnishing by way of the bloodstream a proper combination of nutrients to all other organs and tissues. The liver thus warrants special attention in a discussion of tissue-specific metabolism.

Figures 7.4, 7.5, and 7.6 illustrate the fate of glucose 6-phosphate, amino acids, and fatty acids, respectively, in the liver. In these figures, anabolic pathways are shown pointing upward; catabolic pathways, pointing down; and distribution to other tissues, running horizontally. The pathways indicated are described in detail in Chapters 3, 5, and 6, which deal with carbohydrate, lipid, and protein metabolism, respectively.

Glucose entering the hepatocytes is phosphorylated by glucokinase to glucose 6-phosphate. Other dietary monosaccharides (fructose, galactose, and mannose) are also phosphorylated and rearranged to glucose 6-phosphate. Figure 7.4 shows the possible metabolic routes available to glucose 6-phosphate. Liver glycogenesis likely occurs primarily from newly synthesized glucose derived from gluconeogenic precursors delivered to the hepatocytes from peripheral tissues, rather than through preformed glucose directly [3] (Figure 3.13). This finding is referred to again in the following section.

Figure 7.5 reviews the particularly active role of the liver in amino acid metabolism. The liver is the site of synthesis of many different proteins, both structural and plasma-borne, from amino acids. Amino acids also can be converted in the liver into nonprotein products such as nucleotides, hormones, and porphyrins. Catabolism of amino acids can take place in the liver, where most are transaminated and degraded to acetyl CoA and other TCA cycle intermediates. These substances in turn can be oxidized for energy or converted to glucose or fat. Glucose formed from gluconeogenesis can be transported to muscle for use by that tissue. Newly synthesized fatty acids can be mobilized to adipose tissue for storage or used as fuel by muscle. Hepatocytes are the exclusive site for the formation of urea, the major excretory form of amino acid nitrogen.

The fate of fatty acids entering the liver is outlined in Figure 7.6. Fatty acids can be assembled into liver triacylglycerols or released into the circulation as plasma lipoproteins. In humans, most fatty acid synthesis takes place in the liver rather than in adipocytes. Adipocytes store triacylglycerols arriving from the liver, primarily in the form of plasma VLDLs, and from the lipoprotein lipase action on chylomicrons. Under most circumstances, fatty acids are the major fuel supplying energy to the liver by oxidation. The acetyl CoA that cannot be used for energy can be used for the formation of the ketone bodies, which are very important fuels for certain peripheral tissues such as the brain and heart muscle, particularly during periods of prolonged fasting.

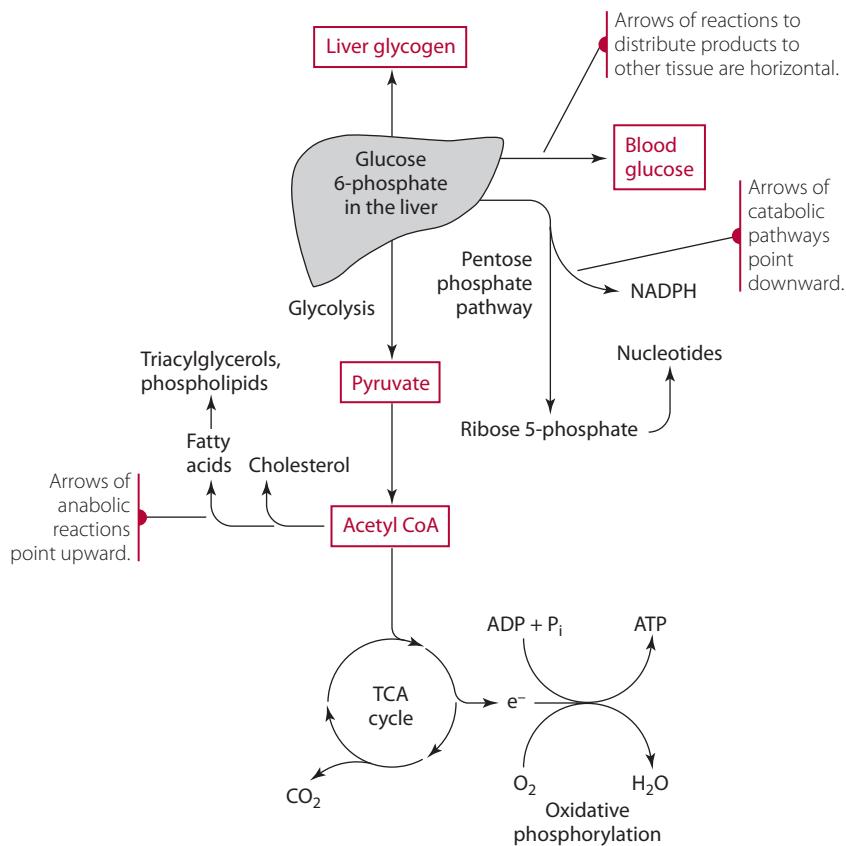


Figure 7.4 Metabolic pathways for glucose 6-phosphate in the liver.

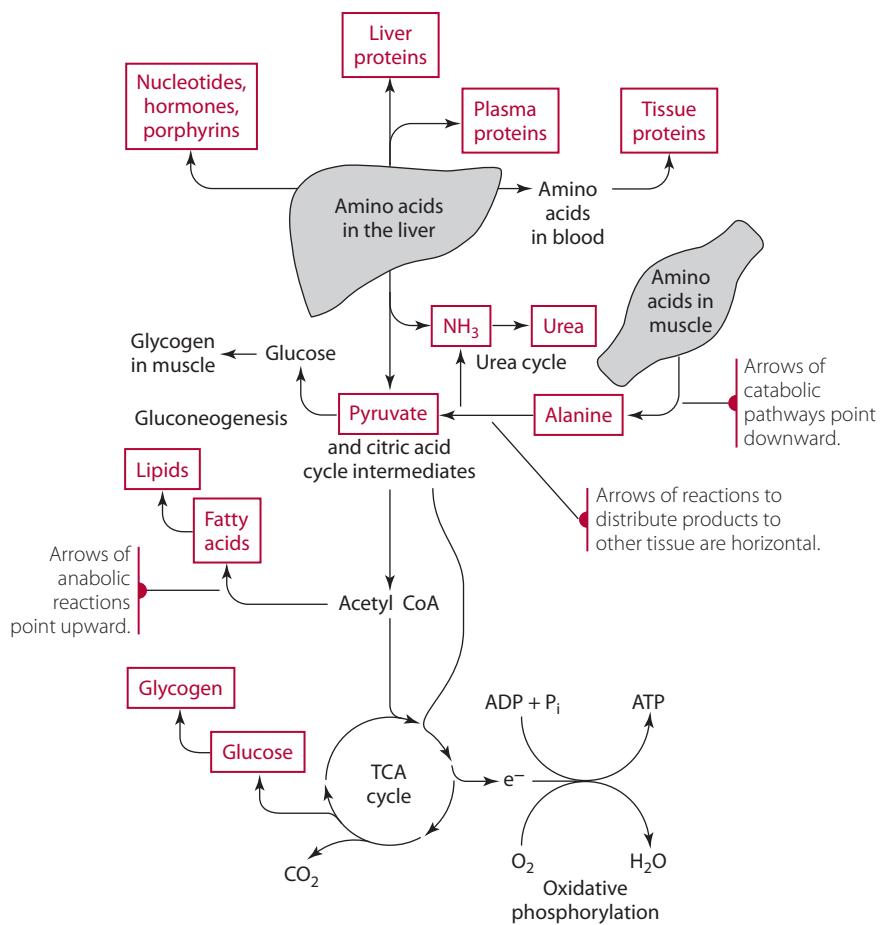


Figure 7.5 Pathways of amino acid metabolism in the liver.

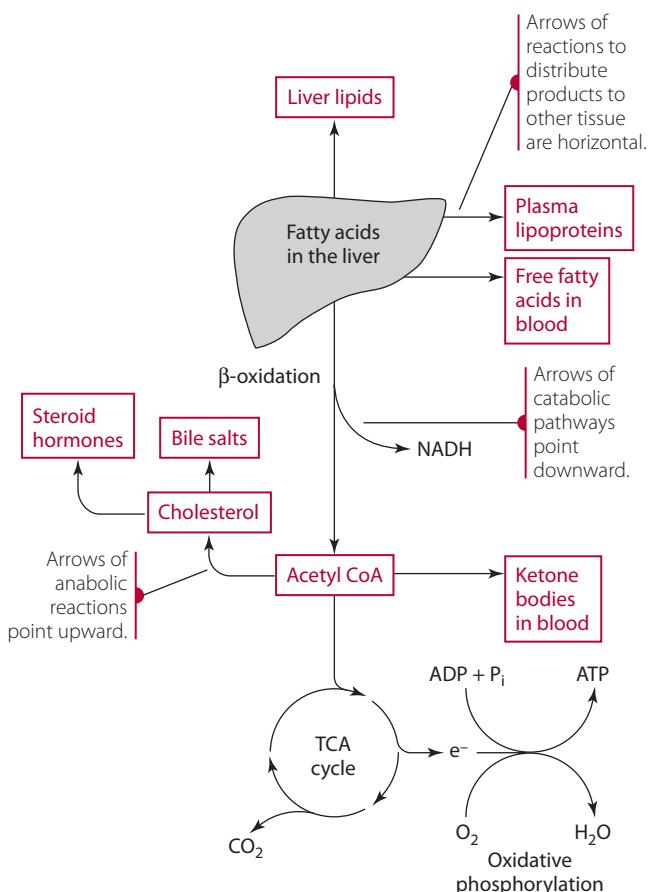


Figure 7.6 Pathways of fatty acid metabolism in the liver.

Tissue-Specific Metabolism during the Fed-Fast Cycle

CARBOHYDRATE AND LIPID METABOLISM

The best way to appreciate the interrelationship of metabolic pathways and the involvement of different organs and tissues in metabolism is to gain an understanding of the fed-fast cycle. The human typically eats specific meals followed by a period of not eating. Food consumption is often 100 times greater than basic caloric needs during the short period of time the meal is consumed, allowing humans to survive from meal to meal without nibbling continuously. Because glucose is a major fuel for tissues, it is very important that glucose homeostasis be maintained, whether food has just been consumed or a state of fasting exists. If the period since the last meal is short (less than 18 hours), the mechanisms used to maintain glucose homeostasis are different from those used if the fasting state is prolonged. During prolonged fasts, other fuels gain importance. The extent to which different organs are involved in carbohydrate and fat metabolism varies within the fed-fast cycles that underlie the eating habits of the human being. When energy consumption exceeds expenditures, the excess calories are

stored as glycogen and fat, which can be used as needed. A fed-fast cycle can be divided into four states, or phases:

- the fed state, which lasts about 3 hours after a meal is ingested
- the postabsorptive or early fasting state, which occurs from about 3 hours to about 12–18 hours following the meal
- the fasting state, which lasts from about 18 hours up to about 2 days without additional intake of food
- the starvation state or long-term fast, a fully adapted state of food deprivation that lasts as long as several weeks

Clearly, in a normal eating routine only the fed and postabsorptive (early fasting) states apply. The time frames of the phases cited are only approximate and are strongly influenced by factors such as activity level, the caloric value and nutrient composition of the meal, and the subject's metabolic rate.

The Fed State

Figure 7.7 illustrates the disposition of glucose, fat, and amino acids among the various tissues during the fed state. The red blood cells (RBCs) do not have mitochondria and can burn glucose only anaerobically. The central nervous system (CNS) has no metabolic mechanisms by which glucose can be converted to energy stores, nor can it make glycogen or store triacylglycerols. Glucose available to these tissues is oxidized immediately to produce energy. In the liver, in contrast, some glucose can be converted directly to glycogen. Contrary to the conventional view of liver glycogenesis, however, research indicates that most liver glycogen is synthesized indirectly from gluconeogenic precursors (pyruvate, alanine, and lactate) returning to the liver from the periphery rather than directly from glucose entering the liver by way of the portal vein [3]. A likely source of lactate for the liver is the red blood cells, as indicated in Figure 7.7. The reason that glucose is not used well as a direct precursor of glycogen has been attributed to the low phosphorylating activity of the liver at physiological concentrations of glucose [4].

The liver is the first tissue to have the opportunity to use dietary glucose. In the liver, glucose can be converted to glycogen. When available glucose or its gluconeogenic precursors exceed the glycogen storage capacity of the liver, the excess glucose can be metabolized in a variety of ways, as shown in Figure 7.4 and in somewhat more detail in Figure 7.7. The conversion of glucose to glycogen and fatty acids is important because both represent the storage of glucose carbon. The conversion of glucose to fatty acids appears to occur only if energy intake exceeds energy expenditure. The potential conversion of excess glucose to fatty acids is particularly crucial because these fatty acids, along with those removed from the chylomicrons and VLDL by lipoprotein lipase, can be stored in the adipose

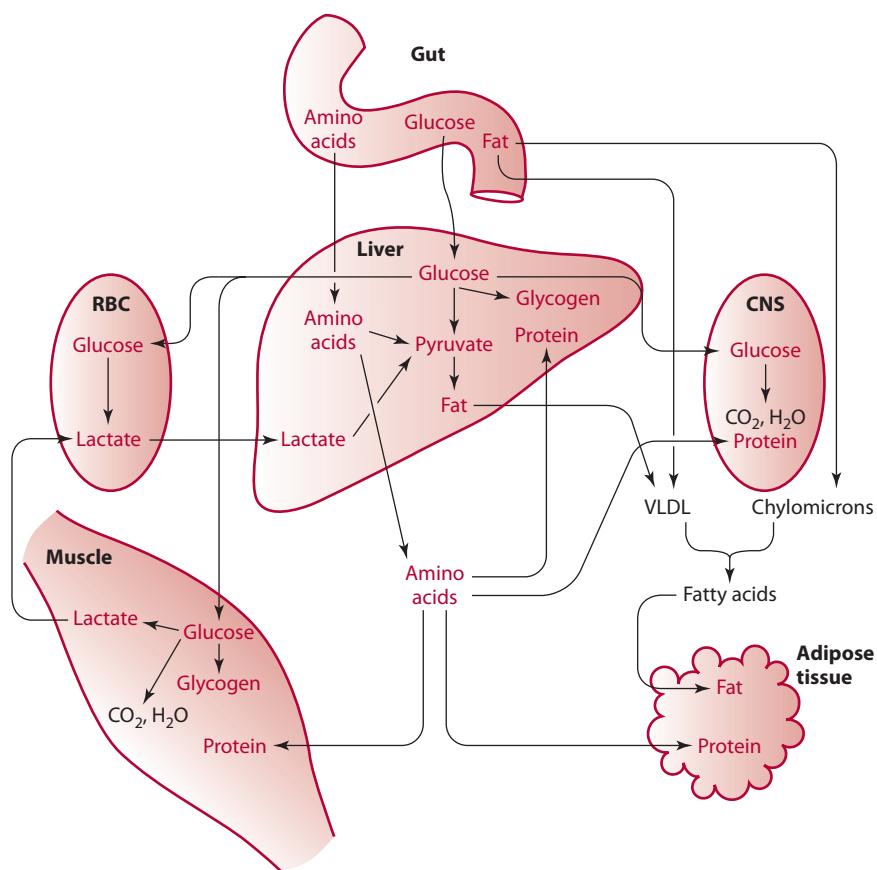


Figure 7.7 Disposition of dietary glucose, amino acids, and fat in the fed state.

tissue, thereby providing a ready source of fuel for most body tissues during the postabsorptive and fasting states.

Some exogenous glucose—that portion coming from the intestine—bypasses the liver and circulates to other tissues. The brain and other tissues of the central nervous system are almost solely dependent on glucose as an energy source during the fed and postabsorptive states. Other major users of glucose include:

- the RBCs, which, lacking mitochondria, convert glucose by way of the glycolytic pathway to lactate for the small amount of energy the cell requires and also use glucose as a source of NADPH through the hexosemonophosphate shunt
- adipose tissue, which can use glucose to some extent as a precursor for both the glycerol and the fatty acid components of triacylglycerols (although most triacylglycerols are synthesized by the liver and transported to the adipose tissue)
- muscle, which uses glucose for the synthesis of glycogen and for the production of energy

With the exception of the RBCs, all the tissues included in Figure 7.7 actively catabolize glucose for energy by glycolysis and the TCA cycle.

In considering fat delivery to the tissues, it is necessary to differentiate between exogenous and endogenous fat. Dietary fat, except for short-chain fatty acids, enters the

bloodstream as chylomicrons, which are promptly acted on by lipoprotein lipase from the vascular endothelium, releasing free fatty acids and glycerol (Chapter 5). Chylomicron remnants remaining from this hydrolysis are taken up by the liver, and their lipid contents are transferred to the very low density lipoprotein fraction. Endogenous fatty acids of the serum are taken up by the adipocytes, reesterified with glycerol to form triacylglycerols, and stored as such as large fat droplets within the cells.

The Postabsorptive or Early Fasting State

With the onset of the postabsorptive state, tissues can no longer derive their energy directly from ingested glucose and other ingested macronutrients but instead must begin to depend on other sources of fuel (Figure 7.8). During the short period of time marking this phase (a few hours after eating), hepatic glycogenolysis is the major provider of glucose to the blood, which serves to deliver it to other tissues for use as fuel. When glycogenolysis is occurring, the synthesis of glycogen and triacylglycerols in the liver is diminished, and the de novo synthesis of glucose (gluconeogenesis) begins to help maintain blood glucose levels.

Lactate, formed in and released by RBCs and muscle tissue, becomes an important carbon source for hepatic gluconeogenesis. The glucose-alanine cycle, in which carbon in the form of alanine returns to the liver from muscle cells, also becomes important. The alanine is then converted

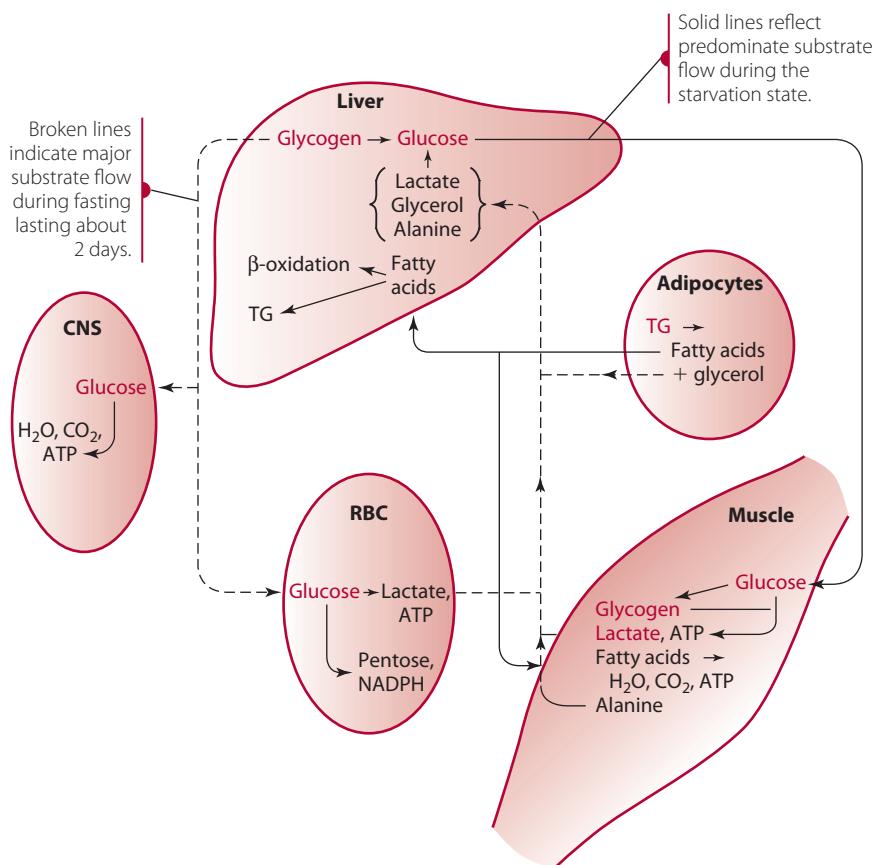


Figure 7.8 The primary postabsorptive flow of substrates among the liver, CNS, adipose tissue, muscle, and red blood cells. Source: Modified from Zakim D, Boyer T, eds. *Hepatology: A Textbook of Liver Disease*, 4th ed. Philadelphia: WB Saunders, 2003.

to pyruvate as the first step in the gluconeogenic conversion of alanine in the liver. The alanine cannot be converted to glucose in the muscle. In the postabsorptive state, glucose provided to the muscle by the liver comes primarily from the recycling of lactate and alanine and to a lesser extent from hepatic glycogenolysis. Muscle glycogenolysis provides glucose as fuel only for muscle cells in which the glycogen is stored, because muscle lacks the enzyme glucose 6-phosphatase. Once phosphorylated in the muscle, glucose is trapped there and cannot leave except as three-carbon units of lactate or alanine.

The brain and other tissues of the CNS are extravagant consumers of glucose, oxidizing it for energy and releasing no gluconeogenic precursors in return. The rate of glucose use is greater than the rate of glucose production by gluconeogenesis, and the stores of liver glycogen begin to diminish rapidly. In the course of an overnight fast, nearly all reserves of liver glycogen and most muscle glycogen have been depleted. Figure 7.8 shows the shifts of metabolic pathways that occur in the tissues during the postabsorptive state.

The Fasting State

The postabsorptive state evolves into the early fasting state after 18 to 48 hours of no food intake. Particularly notable in the liver is the de novo glucose synthesis (gluconeogenesis) that occurs in the wake of glycogen depletion (Figure 7.9). Amino acids from muscle protein breakdown provide the chief substrate for gluconeogenesis, although the glycerol

from lipolysis and the lactate from anaerobic metabolism of glucose also are used to some extent.

The shift to gluconeogenesis during prolonged fasting is signaled by the secretion of the hormone glucagon and the glucocorticosteroid hormones in response to low levels of blood glucose. Proteins are hydrolyzed in muscle cells at an accelerated rate to provide the glucogenic amino acids. Of all the amino acids, only leucine and lysine cannot contribute at all to gluconeogenesis because, as noted previously, these amino acids are totally ketogenic. However, ketogenic amino acids released by muscle protein hydrolysis serve a purpose as well. Because they are converted into ketones—that is, acetyl CoA, acetoacetyl CoA, or acetoacetate—they allow the brain, heart, and skeletal muscles to adapt to using these substrates if the nutritive state continues to deteriorate into a state of long-term fast or starvation.

The early fasting state is accompanied by large daily losses of nitrogen through the urine, in keeping with the high rate of breakdown of muscle protein and the synthesis of glucose through hepatic gluconeogenesis.

The Starvation State

If the fasting state persists and progresses into a starvation state (often referred to as a long-term fast), a metabolic fuel shift occurs again, this time in an effort to spare body protein. This new priority is justified by the vital physiological importance of body proteins. Proteins that must be conserved for life to continue include antibodies, needed to fight infection; enzymes, which catalyze life-sustaining

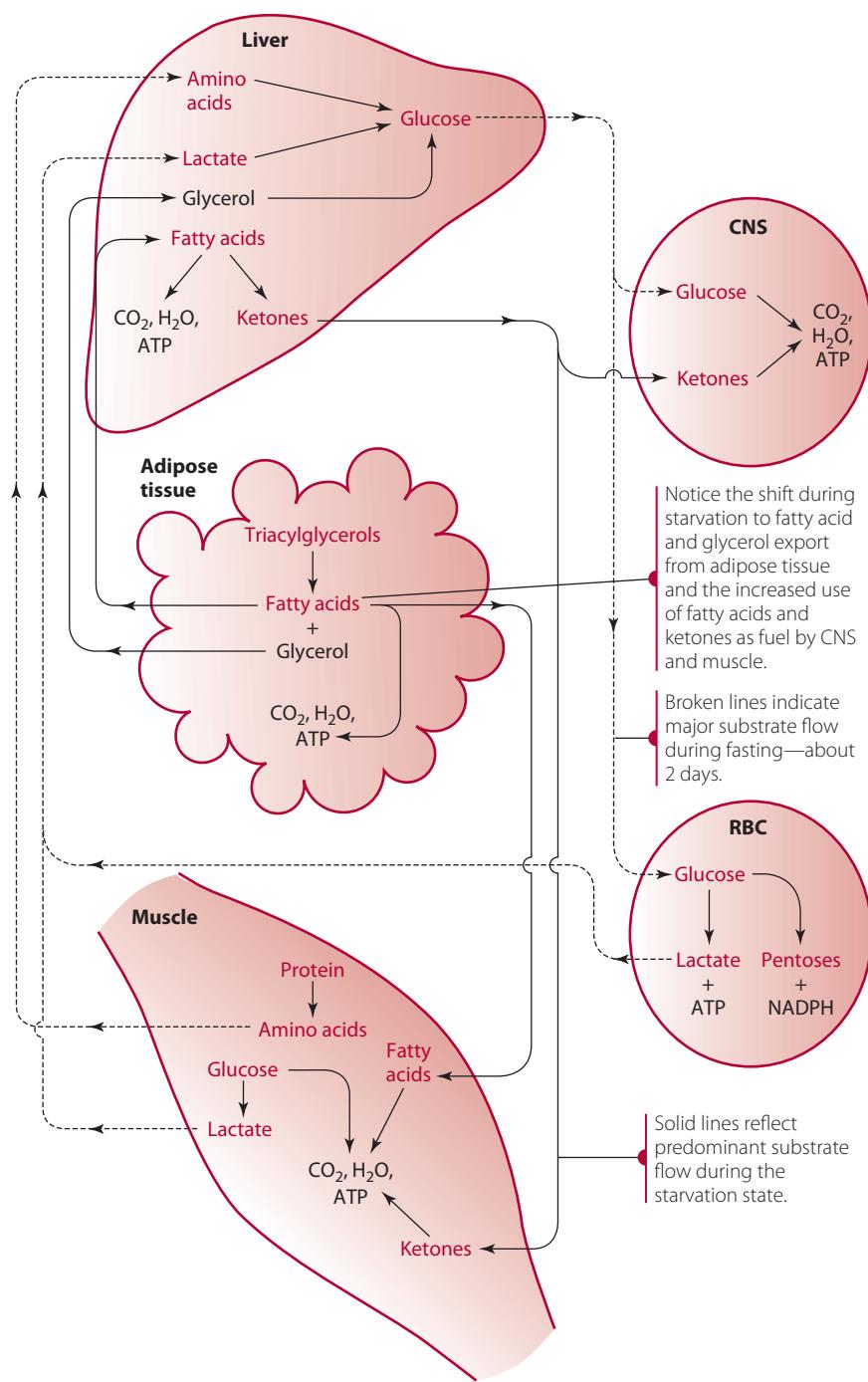


Figure 7.9 Flow of substrates among the liver, CNS, adipose tissue, muscle, and red blood cells during fasting and starvation states.

reactions; and hemoglobin, necessary for the transport of oxygen to tissues. The protein-sparing shift at this point is from gluconeogenesis to lipolysis, as the fat stores become the major supplier of energy. Fat stores, deposited when more calories were consumed than expended, are large in most people. The blood level of fatty acids increases sharply, and fatty acids become the primary fuel for heart, liver, and skeletal muscle tissues that oxidize them for energy. The brain cannot use fatty acids for energy because fatty acids cannot cross the blood-brain barrier. However, the shift to fat breakdown also releases a large amount of glycerol, which replaces amino acids as the major gluconeogenic precursor, assuring a continued supply of glucose as fuel

for the brain. The brain and skeletal muscle also adapt to use ketone bodies for energy.

Eventually, the use of TCA cycle intermediates for gluconeogenesis depletes the supply of oxaloacetate. Low levels of oxaloacetate, coupled with rapid production of acetyl CoA from fatty acid catabolism, cause acetyl CoA to accumulate, favoring formation of acetoacetyl CoA and the ketone bodies. Ketone body concentration in the blood then rises (ketosis) as these fuels are exported from the liver, which cannot use them. They are delivered through the bloodstream to the skeletal muscle, heart, and brain, which oxidize them instead of glucose. As long as ketone bodies are maintained at a high concentration by hepatic

fatty acid oxidation, the need for glucose and gluconeogenesis is reduced, thereby sparing valuable protein. Figure 7.9 illustrates the changes in energy metabolism that occur in various tissues during the fasting and starvation states.

Survival time in starvation depends mostly on the quantity of fat stored before starvation. Stored triacylglycerols in the adipose tissue of a person of normal weight and adiposity can provide enough fuel to sustain basal metabolism for about 3 months. A very obese adult probably has enough fat calories stored to endure a fast of more than

a year, but physiological damage and even death could result from the accompanying extreme ketosis. When fat reserves are gone, the body begins to use essential protein, leading to loss of liver and muscle function and, ultimately, death [5].

AMINO ACID METABOLISM

Organ interactions in amino acid metabolism, illustrated in Figure 7.10, are largely coordinated by the liver. The

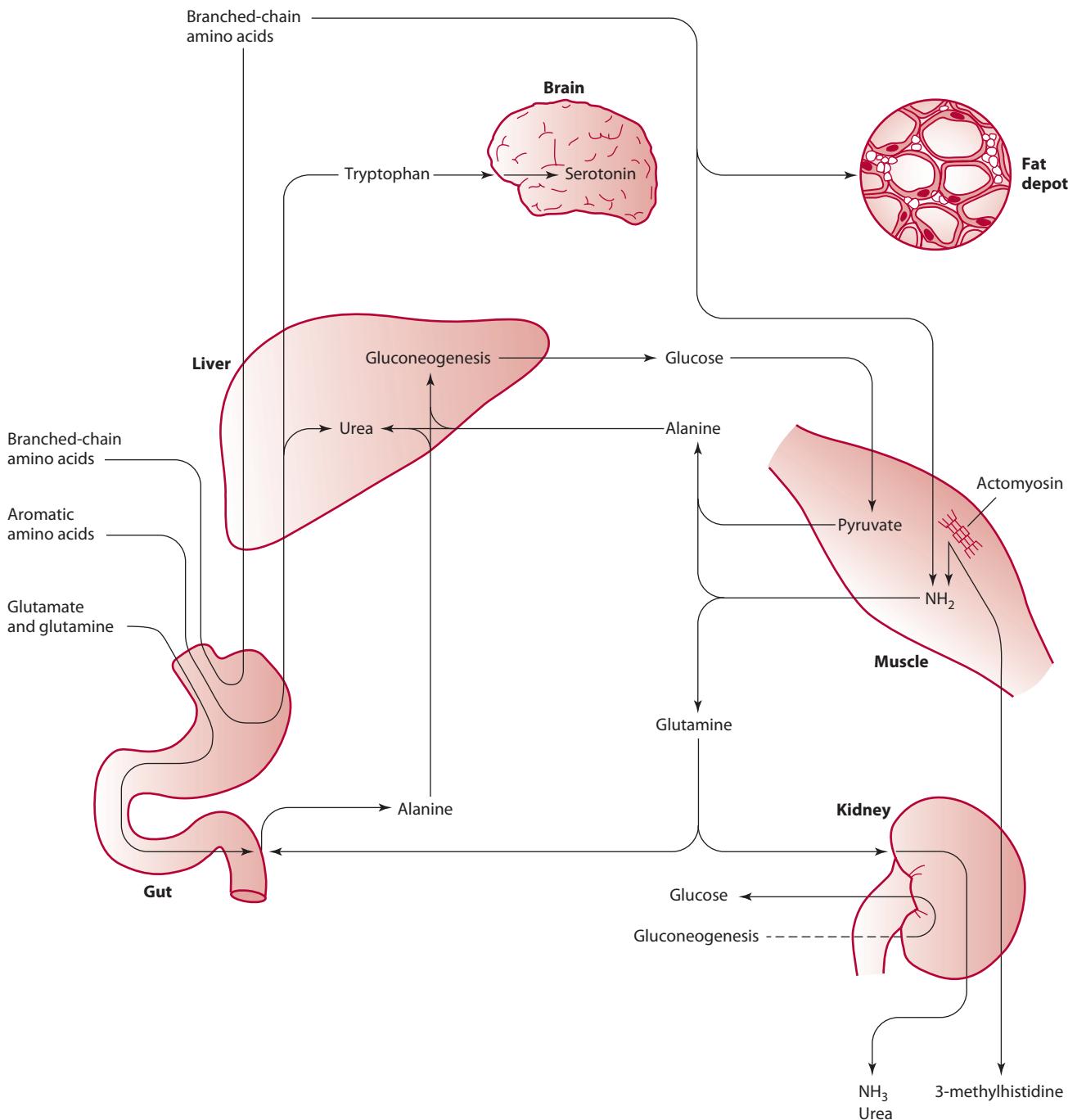


Figure 7.10 Interchanges of selected amino acids and their metabolites among body organs and tissue. Source: Modified from Munro HN. Metabolic integration of organs in health and diseases. *J Parent Enter Nutr* 1982;6:271–79.
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pathways shown undergo regulatory adjustments after consumption of a meal containing protein.

In the fed state, absorbed amino acids pass into the liver, where the fate of most of them is determined in relation to needs of the body. Amounts in excess of need are degraded. Only the branched-chain amino acids (BCAAs) are not regulated by the liver according to the body's needs. Instead, the BCAAs pass to the periphery, primarily to the muscles and adipose tissue, where they may be metabolized. Of particular interest is the fate of the BCAAs that reach the muscle. These amino acids are usually greatly in excess of the amount needed for muscle protein synthesis. The excess is believed to be used to synthesize the dispensable amino acids needed for the increase in protein synthesis that occurs after a protein meal.

The liver is the site of urea synthesis, the primary mechanism for disposing of the excess nitrogen derived from amino acids used for energy or gluconeogenesis. (Chapter 6 describes how urea is formed.) The liver is active in removing the nitrogen from amino acids and uses the α -keto acids (amino acids from which the amine group has been removed) as the chief substrate. During fasting, gluconeogenesis becomes a very important metabolic pathway in regulating plasma glucose levels, and even more nitrogen is available for excretion. Kidney gluconeogenesis is accompanied by the formation and excretion of ammonia.

The importance of the liver to muscle function during the fasting state or during very vigorous exercise is exemplified in the alanine-glucose cycle (Figure 6.35). During periods of fasting or strenuous exercise, the muscle breaks down protein to amino acids. The nitrogen from the amino acids is transaminated to α -ketoglutarate (formed in the TCA cycle) to make glutamic acid. The glutamic acid then transaminates its α -amino group to pyruvate (formed from glycolysis) to make alanine. The alanine enters the bloodstream and is transported to the liver, where it again undergoes transamination. Alanine is converted to pyruvate, and α -ketoglutarate is converted to glutamic acid. This cycle serves several functions. It removes the nitrogen from muscle during a period of high proteolysis and transports it to the liver in the form of alanine. The carbon structure of pyruvate also is transported to the liver, where it can be made into glucose through gluconeogenesis. The synthesized glucose can be transported back to the muscle and used for energy by that tissue. The glucose-alanine cycle also acts as a carrier of amino-nitrogen from intestinal mucosal cells to the liver during periods of amino acid absorption.

Glutamine also plays a central role in transporting and excreting amino acid nitrogen. Many tissues, including the brain, combine ammonia, released primarily by the glutamate dehydrogenase reaction, with glutamate to form glutamine. The reaction is catalyzed by glutamine synthetase. In the form of glutamine, ammonia can then be carried to the liver or kidneys for excretion as urea or ammonium ion,

respectively. In those tissues, glutamine is acted on by the enzyme glutaminase, releasing the ammonia for excretion and re-forming glutamate. Figure 7.10 gives an overview of organ cooperation in these and other aspects of amino acid metabolism. See Chapter 6 for a more detailed discussion of amino acid metabolism in general.

System Integration and Homeostasis

Integration of the metabolic processes, as outlined in the preceding sections, allows the “constancy of the internal milieu” of humans and other multicellular organisms that was described by the French physiologist Claude Bernard about a century ago. This integration of metabolism at the cellular and the organ and tissue levels, which is essential for the survival of the entire organism, receives its direction from body systems. The integration of body systems makes possible communication among all parts of the body.

Three major systems direct activities of the cells, tissues, and organs to ensure their harmony with the whole organism: the nervous, endocrine, and vascular systems.

The **nervous system** is considered the primary communication system because it not only has receiving mechanisms to assess the body's status in relation to its environment but also has transmitting processes to relay appropriate commands to various tissues and organs. The nervous system can inform the body of conditions such as hunger, thirst, pain, and lack of oxygen. This information allows organs to adjust to external changes and may initiate appropriate behavior by the whole organism. Tepperman and Tepperman [6] compare the nervous system to an elaborate system of telegraphy that has a “wire” connection from the source of message initiation to the place where message reception has its needed effect.

The **endocrine system** [6] is compared to a wireless system that transmits messages by way of highly specialized substances called hormones. The endocrine system depends on the vascular system to carry messages to target tissues.

The **vascular system** is comparable to a plumbing system with flexible pipes. It is the primary transport mechanism for the body, not only delivering specialized chemical substances but also carrying oxygen, organic nutrients, and minerals from the external environment to cells throughout the body. It also transports the waste products of metabolism from the cells, carrying them to the lungs and kidneys for elimination.

The concentration of solutes in the blood must be regulated within a narrow range. Among the most prominent sentinel cells that monitor and regulate solute concentration are those that synthesize and secrete hormones. Although hormone synthesis and secretion

occur primarily in the endocrine system, considerable overlap exists between the endocrine system and the CNS. With the discovery of a variety of neuropeptides and recognition of the hormonal action of many of these peptides, it has become apparent that the CNS and the endocrine system are functionally interdependent [6,7]. Tissues and cells that respond to hormones are called the target tissues and target cells of the hormones. These hormone-responsive cells have been preprogrammed by the process of differentiation to respond to the presence of hormones by acting in a predictable way. Not only do hormone-responsive cells respond to hormones through specific receptors, but their metabolic pathways also can be affected by the concentration of available substrates. Hormone-responsive cells live in a complex and continually changing environment of fuels and ions. Their ultimate response to these changes is the net result of both hormonal and nonhormonal information brought to them by the extracellular fluids in which they are bathed [8]. The response of the endocrine system to this information is discussed in the next section.

ENDOCRINE FUNCTION IN FED STATE

Endocrine organs are distributed throughout the body, and most are involved primarily with nutrient ingestion—that is, the gastrointestinal (GI) tract. Interspersed among the absorptive and exocrine secretory cells of the upper GI tract are the highly specialized endocrine cells. These cells present a sensor face to the lumen and secrete granule-stored hormones into the bloodstream. Each of these cells is stimulated to secrete hormones by a different combination of chemical messages. Chemical messages include, for example, glucose, amino acids, fatty acids, and alkaline or acid pH. Hormones secreted by these stimulated GI cells (GIP, CCK, gastrin, secretin) (Table 2.2) then enter the bloodstream and sensitize appropriate cells of the endocrine pancreas for response to the approaching nutrients. The primary action of the GI hormones, secreted in response to a mixed diet, is to amplify the response of the pancreatic islet β -cells to glucose [8].

Insulin, secreted by the β -cells, is the hormone primarily responsible for the direction of energy metabolism during the fed state (Figure 7.7). Its effects can be categorized, based on the time of action, as (1) very fast, occurring in a matter of seconds; (2) fast, occurring in minutes; (3) slower, occurring in minutes to several hours; (4) slowest, occurring only after several hours or even days.

An example of a very fast action of insulin is membrane changes stimulated by the hormone. These changes occur in specific cells, where glucose entry depends on membrane transport (see the section “Glucose Transporters” in Chapter 3). The fast action of insulin involves the activation or inhibition of many enzymes, with anabolic actions accentuated. For example, insulin stimulates glycogenesis,

lipogenesis, and protein synthesis while it inhibits opposing catabolic actions. Several metabolic effects of insulin and the corresponding target enzymes involved are listed in Table 7.1. Insulin favors glycogenesis through the activation of a phosphatase that dephosphorylates phosphorylase and glycogen synthase. This dephosphorylation activates glycogen synthetase while inhibiting the phosphorylase that initiates glycogenolysis. The fast effect of insulin on protein synthesis is not as clear-cut as its influence on lipogenesis and glycogenesis. Nevertheless, protein synthesis is promoted and appears to be related to stimulation of the translation process [8].

One slower action of insulin involves a further regulation of enzyme activity. This regulation is accomplished through the selective induction or repression of enzyme synthesis. The induced enzymes are the key rate-limiting enzymes for anabolic reaction sequences, whereas the repressed enzymes are crucial to the control of opposing catabolic reactions. An example of selective induction is the effect of insulin on glucokinase activity. Insulin increases the synthesis of glucokinase by promoting transcription of the glucokinase gene. Another, slower action of insulin is its stimulation of cellular amino acid influx. The slowest effect of insulin is its promotion of growth through mitogenesis and cell replication. The passage of a cell through its various phases before it can replicate is a relatively slow process that requires 18 to 24 hours to complete.

ENDOCRINE FUNCTION IN POSTABSORPTIVE OR FASTING STATE

Metabolic adjustments that occur in response to food deprivation operate on two time scales: acutely, measured in minutes (such as adjustments operating in a postabsorptive state), and chronically, measured in hours and days (adjustments occurring during fasting or starvation). In contrast to the fed state, in which insulin is the hormone primarily responsible for directing energy metabolism, the body deprived of food requires a variety of hormones to regulate its fuel supply.

Figure 7.8 depicts the postabsorptive state in which hepatic glycogenolysis is providing some glucose to the body while increased use of fatty acids for energy is

Table 7.1 Metabolic Effects of Insulin and Its Action on Specific Enzymes

Metabolic Effect	Target Enzyme
↑Glucose uptake (muscle)	↑Glucose transporter
↑Glucose uptake (liver)	↑Glucokinase
↑Glycogen synthesis (liver, muscle)	↑Glycogen synthase
↓Glycogen breakdown (liver, muscle)	↓Glycogen phosphorylase
↑Glycolysis, acetyl CoA production (liver, muscle)	↑Phosphofructokinase-1
↑Fatty acid synthesis (liver)	↑Pyruvate dehydrogenase complex
↑Triacylglycerol synthesis (adipose tissue)	↑Acetyl CoA carboxylase
	↑Lipoprotein lipase

decreasing the glucose requirement of cells. Also, gluconeogenesis is being initiated, with lactate, glycerol, and alanine serving as substrates.

Hepatic glycogenolysis is initiated through the action of glucagon, which is secreted by the α -cells of the pancreas, and of epinephrine (adrenaline) and norepinephrine, which are synthesized primarily in the adrenal medulla and the sympathetic nerve endings, respectively. Epinephrine is considerably more potent in stimulating glycogenolysis than is norepinephrine, which functions mainly as a neurotransmitter. Epinephrine and norepinephrine are called the catecholamine hormones because they are derivatives of the aromatic alcohol catechol. Although they influence hepatic glycogenolysis somewhat, the catecholamines exert their effect primarily on the muscles. The action of glucagon and the catecholamines is mediated through cAMP and protein kinase phosphorylation. (This mechanism is described in the section on glycogenolysis in Chapter 3; see also Figure 3.16.) Through the action of glucagon on the liver, phosphorylase and glycogen synthetase are phosphorylated, in direct opposition to the action of insulin. Consequently, phosphorylase is activated and glycogen synthetase is inhibited. As a result, glycogen is broken down, giving rise to glucose 6-phosphate, which then can be hydrolyzed by the specific liver phosphatase (glucose 6-phosphatase) to produce free glucose. The free glucose can then enter the bloodstream to maintain blood glucose levels.

Muscle glycogenolysis, in contrast, stimulated by the catecholamines, provides glucose only for use by the particular muscle in which the glycogen has been stored. Phosphorylated glucose cannot cross the cell membrane. Muscle tissue lacks glucose 6-phosphatase and cannot release free glucose into the circulation. The catecholamines, however, raise blood glucose levels indirectly by stimulating the secretion of glucagon and inhibiting the uptake of blood glucose by the muscles.

Glycogenolysis can occur within minutes and thus meets an acute need for raising the blood glucose level. However, because so little glycogen is stored in the liver ($\sim 60\text{--}100\text{ g}$), blood glucose cannot be maintained over a prolonged period. The content of total muscle glycogen is $\sim 350\text{ g}$. Twelve to eighteen hours following a meal, liver glycogen levels are very low. As mentioned previously, gluconeogenesis in the liver is a major supplier of glucose during fasting. Lactate, glycerol, alanine, and other amino acids are the primary precursors. Gluconeogenesis is fostered by the same hormones that initiate glycogenolysis (glucagon and epinephrine), but the amino acids needed as substrates are made available through the action of the glucocorticoids secreted by the adrenal cortex. Glucocorticoid hormones stimulate gluconeogenesis. Alanine, generated in the muscle from other amino acids and from pyruvate by transamination, not only serves as the principal gluconeogenic substrate but also acts as a stimulant of

gluconeogenesis through its effect on the secretion of glucagon. In fact, alanine is the prime stimulator of glucagon secretion by α -cells that have been sensitized to the action of alanine by the glucocorticoids.

Low levels of circulating insulin not only decrease the use of glucose but also promote lipolysis and a rise in free fatty acids. Contributing to this effect is the increase in glucagon during the fasting period. Glucagon raises the level of cAMP in adipose cells, and the cAMP then activates a lipase that hydrolyzes stored triacylglycerols. The muscles, inhibited from taking up glucose by the catecholamines, begin to use fatty acids as the major source of energy. This increased use of fatty acids by the muscles represents an important adaptation to fasting. Growth hormone and the glucocorticoids foster this adaptation because they, like the catecholamines, inhibit in some manner the use of glucose by the muscles.

As starvation is prolonged, less and less glucose is used, thereby reducing the amount of protein that must be catabolized to provide substrate for gluconeogenesis. As glucose use decreases, hepatic ketogenesis increases and the brain adapts to the use of ketones (primarily β -hydroxybutyrate) as a partial source of energy. After 3 days of starvation, about one-third of the energy needs of the brain are met by ketones. With prolonged starvation, ketones become the major fuel source for the brain. Under conditions of continued carbohydrate shortage, ketones are oxidized by the muscles in preference not only to glucose but also to fatty acids. During starvation, the use of ketones by the muscles as the preferred source of energy spares protein, thereby prolonging life. Although Figure 7.9 depicts fuel metabolism during starvation, it does not show some of the adjustments in energy substrates that occur when starvation is prolonged. These adjustments are shown in Table 7.2. As mentioned previously, the duration of starvation compatible with life depends to a large degree on depot fat status.

Table 7.2 Fuel Metabolism in Starvation

Fuel Exchanges and Consumption	Amount Formed or Consumed in 24 hours (g)	
	Day 3	Day 40
Fuel Use by the Brain		
Glucose	100	40
Ketones	50	100
Fuel Mobilization		
Adipose tissue lipolysis	180	180
Muscle protein degradation	75	20
Fuel Output of the Liver		
Glucose	150	80
Ketones	150	150

Source: Adapted from Stryer, L., *Biochemistry*, 3rd ed., p. 640 (New York: Freeman, 1988).

Metabolic Syndrome

Another example of the interrelation of nutrient intake and metabolism is what has been termed *metabolic syndrome*. **Metabolic syndrome** refers to a clustering of a group of risk factors for cardiovascular disease (CVD), chronic kidney disease, and type 2 diabetes. The definition of metabolic syndrome has evolved over the past few years. The multiple definitions have included insulin resistance or glucose intolerance, hypertension, dyslipidemia, and central obesity. Additionally, hyperleptinemia (elevated levels of leptin in the blood, see Chapter 8) and hyperuricemia (elevated levels of uric acid in the blood) have often been included as part of the syndrome. Note that this condition is called *metabolic syndrome*. Calling it a syndrome means that the condition is not a defined disease entity but is a set of symptoms that occur together. Not all of these symptoms must present in each person to classify that person as having metabolic syndrome. A scientific statement from the American Heart Association and the National Heart, Lung, and Blood Institute of the National Institutes of Health has been published [9] describing the diagnosis and management of metabolic syndrome. The clinical diagnosis is based on a person having any three of five symptoms shown in Table 7.3. The American Diabetes Association and European Association for the Study of Diabetes made a similar statement, with a different conclusion [10]. They stated that although no doubt exists that certain cardiovascular disease risk factors tend to cluster, metabolic syndrome is imprecisely defined, and a lack of certainty persists regarding its pathogenesis. They also noted considerable doubt as to the value of using the diagnosis of metabolic syndrome, rather than individual risk factors, to evaluate the risk of developing CVD. They feel that more research must be completed before patient treatment is based on a diagnosis

of metabolic syndrome. Still other reviews are available for the interested reader [11–13].

The cardiovascular physicians have adopted the term metabolic syndrome to provide the criteria for diagnosis. At this time, whether the diagnosis is clinically important in predicting or treating CVD is unclear. Other terms that have been used to describe this syndrome include syndrome X and insulin resistance syndrome. *Syndrome X* was first used to identify the clustering of these symptoms and has largely been replaced. *Insulin resistance syndrome* considers that the underlying defect that ties all of these symptoms together is insulin resistance. Research in this area is very active, and the reader should monitor current findings. Because insulin resistance is considered by some to be the underlying factor in these syndromes, its mechanisms of action are considered briefly in the next section to provide a basis for understanding future research.

INSULIN RESISTANCE

Much controversy persists in this field but, based on current evidence, the following process appears to occur. Insulin resistance results in hyperinsulinemia (increased blood insulin levels). The pancreas apparently releases more insulin in an effort to maintain normal blood glucose levels. The insulin insensitivity, combined with the elevated insulin levels, results in either elevated fasting blood glucose levels, glucose intolerance, or both. The insensitivity to insulin is primarily seen in muscle and adipose tissue (see Chapters 3 and 5 for details). Insulin-resistant muscle loses its ability to stimulate glucose uptake. In adipose tissue, insulin no longer inhibits free fatty acid release. These observations can explain the elevated blood glucose and free fatty acid levels.

The liver and kidney retain their sensitivity to insulin. The elevated insulin levels stimulate the liver triacylglycerol synthesis (TAG). As a consequence of the elevated TAG synthesis and the VLDL-TAG synthesis and secretion, fasting serum triacylglycerol and VLDL-TAG levels are increased. TAG levels in the liver also increase, resulting in nonalcoholic fatty liver disease. The kidney responds to the elevated insulin levels by increasing renal sodium retention and decreasing uric acid clearance. This response results in an increased prevalence of essential hypertension and higher plasma uric acid concentration.

WEIGHT LOSS AND INSULIN INSENSITIVITY

Not all overweight or obese people have insulin resistance. Therefore, weight loss will not reduce the risk for CVD in all obese people equally. No simple test exists to determine who is insulin resistant and who is not. Fasting insulin levels, fasting plasma glucose levels, and triacylglycerol-HDL-C ratios have all been used as indicators for insulin

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resistance, with varying degrees of success. Considerable evidence demonstrates that if a person loses weight, insulin sensitivity improves. The hyperinsulinemia does not prevent weight from being lost. Energy balance and a discussion of different proportions of macronutrients in weight loss diets are covered in Chapter 8. However, variations in the macronutrient content of isocaloric diets have little effect on the improving insulin sensitivity. One common diet for weight loss is to lower the lipid content of the diet and replace it with carbohydrate. The problem with a low-fat, high-carbohydrate diet for a person with insulin resistance is that the additional carbohydrate requires more insulin to be secreted from the pancreas to maintain glucose homeostasis. If the person is insulin resistant, and the pancreas has the capacity, insulin levels will be elevated further.

The increasing prevalence of overweight and obese people makes the study of metabolic syndrome, insulin resistance, and obesity an important consideration for those studying nutrition. The study of the effectiveness of changing diet, lifestyle, and exercise patterns in decreasing the mortality and morbidity in people with metabolic syndrome as they age will be an active area of research and practice for the future.

Sports Nutrition

Humans have courted the challenge of athletic performance and competition since the days of the early Greeks. The science of nutrition emerged much later, spurred by the expanding knowledge of metabolism and the biochemistry on which it is based. Because the energy for physical performance must be derived from nutrient intake, it was only a matter of time before these areas of interest would be linked. The heavy emphasis on the enhancement of health and physical performance in today's society has led sports nutrition to emerge as an important science. Nutrition, as a means of positively affecting physical performance, has become a topic of great interest to all those involved in human performance, the scientist as well as the athlete and athletic trainer.

The human body converts the potential energy of nutrients to usable chemical energy, part of which drives muscle contraction, a process fundamental to athletic prowess. Fluctuations in the body's demand for energy—for example, changes in exertion level among resting, mild exercise, and strenuous exercise—are accompanied by shifts in the rate of catabolism of the different stored forms of nutrients. It follows that an understanding of sports nutrition requires an understanding of the integration of the metabolic pathways that furnish the needed energy. In this respect, therefore, the energy demands of sport resemble the fed-fast cycle described earlier in this

chapter, so a discussion of sports nutrition at this point in the text seems appropriate.

BIOCHEMICAL ASSESSMENT OF PHYSICAL EXERTION

To fully understand sports nutrition, we need to examine different types of skeletal muscle. A more detailed discussion of this topic can be found in an exercise physiology text (such as [8]). Muscle generally is classified as one of three distinct types, each emphasizing a different metabolic pathway: Type I, Type IIa, and Type IIb. Type I muscle, sometimes called red muscle, is oxidative and red in color. It has a large number of mitochondria and therefore is capable of oxidizing glucose to CO_2 and H_2O and carrying out β -oxidation of fatty acids. This muscle typically is used for aerobic endurance events. Type IIa muscle and Type IIb muscle have been called white muscle. Type IIb has fewer mitochondria, has a very active glycolytic pathway, and is white in appearance. This type of muscle is used primarily for short-duration anaerobic events and power events. Type IIa muscle can be considered a hybrid of Type I and Type IIb muscle, with some characteristics of each. Endurance training can make Type IIa muscle act more like Type I muscle, whereas strength training or sprint training can make it look more like Type IIb. Much more could be said about the muscle types and their response to nervous system stimulation and training, but this brief description provides sufficient information to foster an understanding of the resemblance of sports nutrition to the fed-fast cycle. The portion (relative number) of each type of muscle fibers a person has is defined by genetics. Training can increase the size (volume) of a muscle fiber type but does not alter the actual number of fibers of that type. Because some sports rely on a specific muscle type, some people are genetically better fit for a specific type of sport activity based on their muscle type makeup. Interestingly, women have more Type I muscle than men. The result of that difference is that, under usual conditions of long-term aerobic exercise, women burn lipid at higher percentages of $\text{VO}_2 \text{ max}$ than do men [14].

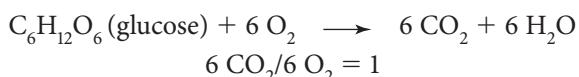
To understand how the muscle types relate to physical exercise at the cellular level, we need to examine two common measurements used by the exercise physiologist [15]: the **respiratory quotient (RQ)** and the **maximal oxygen consumption ($\text{VO}_2 \text{ max}$)**. Respiratory quotient is called the respiratory exchange ratio (R or RER) by exercise physiologists. It is the ratio of CO_2 production to O_2 consumption. Typical RQs for carbohydrate, fat, and protein are 1.0, 0.70, and 0.82, respectively (values are explained below). A newer generation of procedures (e.g., the isotope infusion method) has been developed to measure the relative contribution of substrates to energy supply during exercise. These measurements are described briefly here and more fully in Chapter 8. Further details of how the duration and

intensity of physical conditioning influence which muscle cell type is used, and which metabolic pathways are active, are discussed later in this chapter.

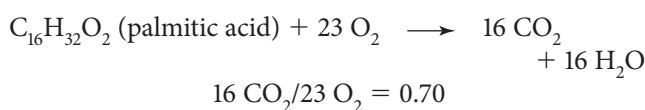
The respiratory quotient (RQ) has served for nearly a century as the basis for determining the relative participation of carbohydrates and fats in exercise [16,17].

$$\text{RQ} = \text{CO}_2/\text{O}_2$$

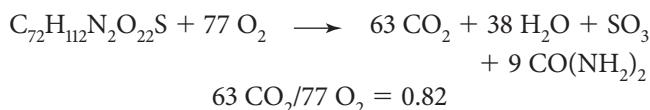
For carbohydrate catabolism, the RQ is 1:



For fat catabolism, the RQ is approximately 0.7:



The RQ for protein is about 0.82:



The amount of protein being oxidized can be estimated from the amount of urinary nitrogen produced, and the remainder of the metabolic energy must made up of a combination of carbohydrate and fat. Should the principal fuel source shift from mainly fat to carbohydrate, the RQ correspondingly increases, and a shift from carbohydrate to fat lowers the RQ. Tables exist that permit the estimation of the relative percentage of either carbohydrate or lipid being used as a metabolic fuel based on the RQ at any given time (for short-term exercise activities, it is often assumed that no amino acids are used for energy). During the past 20 years, however, such knowledge has been advanced by invasive techniques such as arteriovenous measurements and the use of needle biopsies to quantify tissue stores of the energy nutrients. These measurements are used clinically to evaluate elevated rates of metabolism.

The concept of maximum oxygen ($\text{VO}_2 \text{ max}$) uptake is fundamental. As work increases in intensity, the volume of oxygen taken up by the body also increases. The $\text{VO}_2 \text{ max}$ is defined as the point at which a further increase in the intensity of the exercise no longer results in an increase in the volume of oxygen uptake. The intensity level of a particular workload is most commonly expressed in terms of the percentage of the $\text{VO}_2 \text{ max}$ that it induces. As we discuss later, the metabolic pathway that supplies energy for work is determined by the availability of metabolic energy (carbohydrate or lipid) and oxygen as well as by the duration of the activity and the conditioned state of the person performing the work. As a person goes from an untrained state to a trained state the $\text{VO}_2 \text{ max}$ increases. Isotope infusion can be used to quantify the contribution of the major energy substrates, plasma glucose and fatty acids, and muscle triacylglycerols and glycogen to energy expenditure

during exercise. It involves the intravenous infusion of stable isotope (e.g., ^2H [deuterium]-labeled glucose, palmitate, and glycerol during periods of rest and exercise. By monitoring the uptake of infused labeled glucose and palmitate and knowing whole-body substrate oxidation, the contribution of muscle triacylglycerol and glycogen to overall energy supply can be estimated [15].

ENERGY SOURCES DURING EXERCISE

The hydrolysis of the terminal phosphate group of ATP ultimately provides the energy for conducting biological work. In terms of physical performance, the form of work that is of greatest interest is the mechanical contraction of skeletal muscles. The physical exertion depends on a reservoir of ATP, which is in an ever-changing state of metabolic turnover. Whereas ATP is consumed by physical exertion, its stores are supplemented by the metabolic pathway discussed next and are repleted during periods of rest. The key to optimizing physical performance lies in nutritional strategies that maximize cellular levels of stored nutrients as fuels for ATP production. Three energy systems supply ATP during different forms of exercise [17]:

- the ATP-CP (creatine phosphate) system
- the lactic acid system (anaerobic glycolysis)
- the aerobic system (aerobic glycolysis, TCA cycle, and β -oxidation of fatty acids)

The ATP-CP (Phosphagen) System The ATP-CP system is a cooperative system in muscle cells using the high-energy phosphate bond of creatine phosphate (CP) together with ATP (Chapter 3). When the body is at rest, energy needs are fulfilled by aerobic catabolism (see the section “The Aerobic System” in this chapter) because the low demand for oxygen can easily be met by oxygen exchange in the lungs and by the oxygen carried to the muscle by the cardiovascular system. (The ATP-CP system also operates continuously during this time, though at a slow pace.) If physical activity is initiated, the energy requirements of contracting muscle are met by existing ATP. However, stores of ATP in muscle are limited, providing enough energy for only a few seconds of maximal exercise. As ATP levels diminish, they are replenished rapidly by the transfer of high-energy phosphate from creatine phosphate (CP) to form ATP in the ATP-CP system. The muscle cell concentration of CP is only four to five times greater than that of ATP, and therefore all energy furnished by this system is expended after approximately 10 to 25 seconds of strenuous exercise. When the ATP-CP is expended, the lactic acid system (anaerobic glycolysis) kicks in to produce more ATP. Performance demands of high intensity and short duration such as weightlifting, 100 m sprinting, some positions in football, and various short-duration field events benefit most from the ATP-CP system. Lower-intensity activity may allow a person to use this system for up to 3 minutes.

The Lactic Acid System This system involves the glycolytic pathway by which ATP is produced in skeletal muscle by the incomplete breakdown of glucose anaerobically into 2 mol of lactate. The source of glucose is primarily muscle glycogen and, to a lesser extent, circulating glucose, and the lactic acid system can generate ATP quickly for high-intensity exercise. As pointed out in Chapter 3, the lactate system is not efficient from the standpoint of the quantity of ATP produced. However, because the process is so rapid, the small amount of ATP is produced quickly and absolutely by substrate-level phosphorylation of ADP. The lactate produced by this system quickly crosses the muscle cell membrane into the bloodstream, from which it can be cleared by other tissues (primarily the liver) for aerobic production of ATP or gluconeogenesis. If the rate of production of lactate exceeds its rate of clearance by the liver, blood lactic acid accumulates. This accumulation lowers the pH of the blood and is one cause of fatigue. Under such circumstances, exercise cannot be continued for long periods. The lactic acid system is engaged to provide a rapid source of energy. When an inadequate supply of oxygen prevents the aerobic system from furnishing sufficient ATP to meet the demands of exercise, the lactic acid system will continue to function. Although the lactic acid system is operative as soon as strenuous exercise begins, it becomes the primary supplier of energy only after CP stores in the muscle are depleted. As a backup to the ATP-CP system, the lactic acid system becomes very important in high-intensity anaerobic power events that last from 20 seconds to a few minutes, such as sprints of up to 800 m and swimming events of 100 or 200 m.

The Aerobic System This system involves the TCA cycle, through which carbohydrates, fats, and some amino acids are completely oxidized to CO_2 and H_2O . The system, which requires oxygen, is highly efficient from the standpoint of the quantity of ATP produced. Because oxygen is necessary for the system to function, a person's $\text{VO}_2 \text{ max}$ becomes an important factor in performance capacity. Contributing to the $\text{VO}_2 \text{ max}$ are the cardiovascular system's ability to deliver blood (which carries the oxygen) to exercising muscle, pulmonary ventilation, oxygenation of hemoglobin, release of oxygen from hemoglobin at the muscle, and use of the oxygen by skeletal muscle mitochondria. Matching these contributors to the cellular need for oxygen in exercising muscle is complex, because low efficiency of any of them becomes rate limiting for the entire process. In terms of cellular metabolism, the aerobic pathway is slow to become activated and begins to dominate the course of activity only after about 5 minutes of continuous activity. The aerobic system is an important supplier of energy for forms of exercise lasting longer than 3 or 4 minutes, depending on the intensity of the exercise. Both intracellular triacylglycerols and plasma fatty acids also contribute to the overall energy supply. Many types of exercise or sports meet these criteria,

for example, distance running, distance swimming, and cross-country skiing, just a few of the so-called endurance feats.

Current thinking is that the three energy systems do not simply take turns serially, and that no particular system is skipped in meeting the demands of exercise. Rather, all systems function at all times, and as one predominates, the others participate to varying degrees. The interaction of the three systems over the course of the first 2 minutes of exercise is complex but appears to involve the following energy contributions: ATP-CP system initially supplies energy, and as ATP begins to be depleted after 10 seconds or so, the lactic acid system phases in and becomes the major supplier of energy. After 3 minutes or so, aerobic glycolysis starts to be the major supplier energy. It takes about 20 minutes of moderate exercise for fatty acids to be a major contributor. Energy contributions to long-term activity from aerobic and anaerobic systems are shown in Figure 7.11.

Fuel Sources during Exercise Carbohydrate, fat, and protein are the dietary sources that provide the fuel for energy transformation in the muscle. At rest, and during normal daily activities, fats are the primary source of energy, providing 80% to 90% of the energy. Carbohydrates provide 5% to 18%, and protein provides 2% to 5% of energy during the resting state [18].

During exercise, the oxidation of amino acids contributes only minimally to the total amount of ATP used by working muscles. Significant breakdown of amino acids occurs only toward the end of a long endurance event, when carbohydrate (glycogen) stores are somewhat depleted. Amino acids can be transaminated to form alanine from pyruvate. The alanine is transported to the liver and is a primary substrate for gluconeogenesis. This process is termed the glucose-alanine cycle or Cori cycle and is described in Chapter 3. The carbon skeleton of some amino acids can be oxidized directly in the muscle. During exercise, the four major endogenous sources of energy are:

- muscle glycogen
- plasma glucose
- plasma fatty acids
- intramuscular triacylglycerols

The extent to which each of these substrates contributes energy for exercise depends on several factors, including:

- the intensity and duration of exercise
- the level of exercise training
- initial muscle glycogen levels
- supplementation with carbohydrates through the intestinal tract during exercise

This section describes the relationship between these factors and the “substrate of choice” for energy supply. A graphical representation of the contribution of these

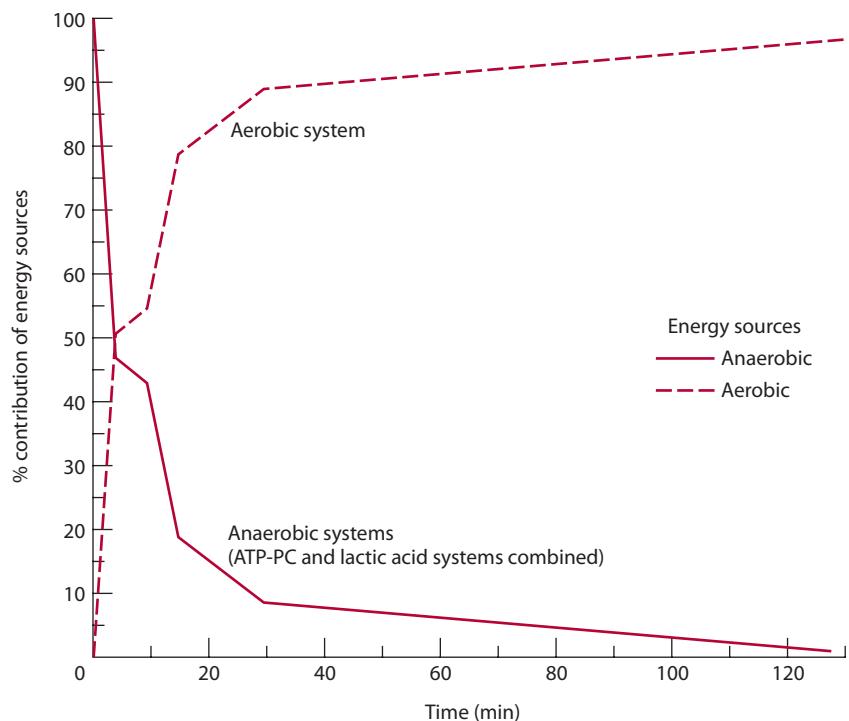


Figure 7.11 Primary energy sources for long-duration activity. Source: Adapted from Fox EL, Bowers RW, Foss ML. *The Physiological Basis for Exercise and Sports*, 3rd ed. Dubuque, IA: Brown and Benchmark, 1989, p. 37. Reproduced with permission of The McGraw-Hill Companies.

substrates at 25%, 65%, and 85% VO_2 max is shown in Figure 7.12.

Exercise Intensity and Duration In the fasting state, much of the energy required for low intensity levels of exercise (25%–30% VO_2 max) is derived from muscle triacylglycerols and plasma fatty acid oxidation, with a small contribution from plasma glucose. The pattern does not change

significantly over a period of up to 2 hours at this exercise level, which is equivalent to walking. During this time, the consumed plasma fatty acids are replaced by fatty acids mobilized from the large triacylglycerol stores in adipocytes throughout the body. However, as exercise intensity increases to 65% and up to 85% VO_2 max, fewer adipocyte fatty acids are released into the plasma, resulting in a decreased concentration of plasma fatty acids. This decrease occurs despite a continuing high rate of lipolysis in adipocytes. The decreased replacement of plasma fatty acids from fat stores at higher levels of exercise has been attributed to insufficient blood flow and albumin delivery of fatty acids from adipose tissue into the systemic circulation [19]. Therefore, we would predict that fatty acids become trapped in adipose tissue and accumulate there during high levels of exercise, a theory supported by research [15].

With moderate-intensity exercise (~65% VO_2 max) equivalent to running for 1 to 3 hours, total fat oxidation increases, despite the reduced rate of return of adipose fatty acids into the circulation. This increase is attributed to an increase in the oxidation of muscle triacylglycerols. In fact, as shown in Figure 7.12, plasma fatty acids and muscle triacylglycerols contribute equally to energy expenditure at this level of exertion in endurance-trained athletes. Within the exertion range of 60% to 75% VO_2 max, however, fat cannot be oxidized at a rate sufficiently rapid to provide needed energy, and therefore nearly half of the required energy must be furnished by carbohydrate oxidation. Note that fatty acids have only two oxygen molecules, compared to carbohydrates' equal number of oxygen and carbon

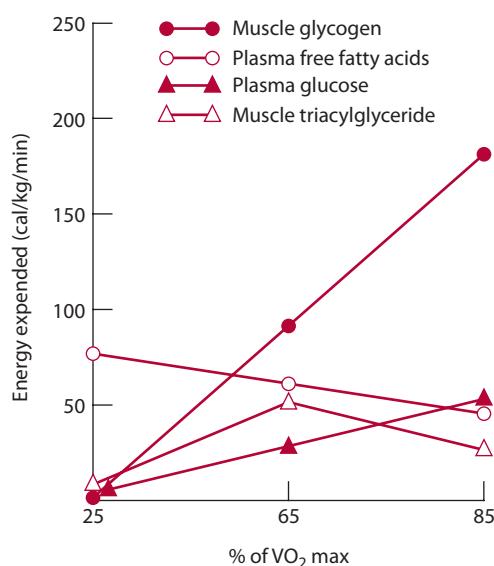


Figure 7.12 Contribution of the four major substrates to energy expenditure after 30 minutes of exercise at 25%, 65%, and 85% VO_2 max.

molecules. This characteristic means that fatty acids require more oxygen to be delivered by the cardiovascular system. Also, the transfer of fatty acids into mitochondria is slow, and this may be a rate-limiting event. The result is that when tissue oxygen levels begin to be low or high-intensity exercise calls for a large quantity of energy, carbohydrate becomes a more favored substrate. Fatty acids are the favored substrates for intensities of up to about 50% VO_2 max.

As exercise intensity increases to 85% VO_2 max, the relative contribution of carbohydrate oxidation to total metabolism increases sharply (Figure 7.12). At VO_2 max, carbohydrate in the form of blood glucose (derived from glycogenolysis of hepatic glycogen stores) and muscle glycogen essentially become the sole suppliers of energy. Like muscle glycogen, the concentration of blood glucose falls progressively during prolonged, strenuous exercise. This decrease occurs because glucose uptake by working muscle (independent of insulin) may increase to as much as 20-fold or more above resting levels, while hepatic glucose output decreases with exercise duration. Interestingly, however, hypoglycemia is not always observed at exhaustion, particularly at exercise intensities >70% VO_2 max. Hypoglycemia following liver glycogen depletion apparently can be postponed by an inhibition of glucose uptake and accelerated gluconeogenesis in the liver, using the glycerol produced in lipolysis, and by lactate and pyruvate (which was carried to the liver as alanine) produced by the glycolytic activity of the working muscles.

Accompanying high rates of carbohydrate catabolism is a rise in the production of lactic acid, which accumulates in muscle and blood. This increase in lactic acid is particularly evident in situations of oxygen debt, in which insufficient oxygen to complete the oxidation of pyruvate to CO_2 and H_2O instead favors its reduction to lactate.

Carbohydrate is an essential energy substrate at moderate to high levels of exercise because of the need for TCA cycle intermediates from carbohydrates to oxidize the fatty acids, the slow rate of fat oxidation, and the limited ability of muscle to oxidize fat at high rates. Muscle fatigue occurs when the supply of glucose is inadequate, such as occurs with muscle glycogen depletion or hypoglycemia. To delay muscle fatigue, the person must reduce workload intensity to a level that matches his or her ability to oxidize fat predominantly, possibly as low as 30% VO_2 max. The reason for this limitation, and thus the dependence of muscle on carbohydrate as an energy source, is not fully understood. However, traditional thinking is that the limitation may be based on two factors: (1) oxidation of fatty acids is limited by the enzyme carnitine acyltransferase (CAT), which catalyzes the transport of fatty acids across the mitochondrial membrane; and (2) CAT is known to be inhibited by malonyl CoA. When availability of carbohydrate to the muscle is high, fatty acid oxidation may be reduced by the inhibition of CAT by glucose-derived malonyl CoA [20].

Level of Exercise Training Endurance training increases an athlete's ability to perform more aerobically at the same absolute exercise intensity. Several factors aid in this increase. Endurance-trained muscle exhibits an increase in the number and size of mitochondria. Cardiovascular and lung capacity also increase, and Type I muscle hypertrophies. This hypertrophy is an increase in the size of the Type I muscle, not the number of muscle fibers. The activity of oxidative enzymes in endurance-trained subjects has been shown to be 100% greater than in untrained subjects at 65% VO_2 max. Endurance training also results in an increased use of fat as an energy source during submaximal exercise. In skeletal muscle, fatty acid oxidation inhibits glucose uptake and glycolysis. For this reason, the trained athlete benefits from the carbohydrate-sparing effect of enhanced fatty acid oxidation during competition, because muscle glycogen and plasma glucose are depleted more slowly. This effect largely accounts for the training-induced increase in endurance for exercise over a prolonged period.

Trained athletes have been reported to have lower plasma fatty acid concentrations and reduced adipose tissue lipolysis than untrained counterparts do at similar exercise intensity. This finding suggests that the primary source of fatty acids used by the trained athlete is intramuscular triacylglycerol stores, rather than adipocyte triacylglycerols. After exercise, the intramuscular triacylglycerols are replaced with the fatty acids coming from plasma. Lipolysis from adipocytes increases the free fatty acid levels in plasma. This process can result in shrinking the size of the adipose tissue.

Endurance training appears to result in an increased capacity for muscle glycogen storage. Therefore, the trained athlete benefits not only from a slower use of muscle glycogen (as explained earlier) but also from the capacity to have higher glycogen stores at the onset of competition.

Initial Muscle Glycogen Levels The ability to sustain prolonged moderate-to-heavy exercise largely depends on the initial content of skeletal muscle glycogen, and the depletion of muscle glycogen is the single most consistently observed factor that contributes to fatigue. High muscle glycogen levels allow exercise to continue longer at a submaximal workload. Even in the absence of carbohydrate loading (see the following section), a strong positive correlation exists between initial glycogen level and time to exhaustion, level of performance, or both during exercise periods that last more than 1 hour. The correlation does not apply at low levels of exertion (25%–35% VO_2 max), or at high levels of exertion for short periods, because glycogen depletion is not a limiting factor under these conditions. It has been suggested that the importance of initial muscle glycogen stores is related to the inability of glucose and fatty acids to cross the cell membrane rapidly enough to provide adequate substrate for mitochondrial respiration [21].

Let's review the changes in the source of energy during long endurance events. If a well-trained person were to

run a marathon (a 26.2 mile run), her source of biological fuel and metabolic pathway to supply the energy would change. These changes do not occur abruptly, but one source (or pathway) begins to decrease while the next one begins to increase. During the first 10 seconds or so, most of the energy is supplied by preformed muscle ATP. As the ATP begins to be used, the CrP-ATP system kicks in and creatine phosphate starts to resupply the ATP. After 20 to 30 seconds, the lactic acid system starts. This system uses muscle glucose in the beginning, followed by a rapid breakdown of muscle glycogen. The anaerobic glycolysis continues for about the next 5 minutes and then metabolism shifts to aerobic metabolism. The glucose comes from muscle glycogen, and from blood glucose absorbed by the muscle fiber by a non-insulin dependent process. During this time, the β -oxidation of fatty acids begins. It takes about 20 minutes into the exercise before fatty acid oxidation proceeds at its maximum rate. Depending upon the level of exertion (the % VO_2 max), this energy can continue for a long time. If the person is well trained, it can last for two to three hours or more. At the midpoint of the marathon the person will be burning aerobically about a 40/60 split between carbohydrate and lipid. Which one dominates depends on the level of training, the level of exertion, the availability of oxygen to the muscle, and the availability of each of the biological fuels. At this point, the lipid comes mostly from muscle triacylglycerol. Once glucose becomes limited, the oxidation of fatty acid cannot continue. Some (but a limited amount) of fatty acids are absorbed from blood from the increased free fatty acids that have been released from adipose tissue. At the midpoint, glucose comes mostly from gluconeogenesis by the liver. During the last phase of the marathon (about the last 10 to 15 minutes), skeletal muscle protein begins to break down. The amino acids transfer their amino group to α -ketoglutarate and then to alanine. The alanine is transported to the liver, where it will be converted to glucose by gluconeogenesis. During this time, branched-chain amino acids (from skeletal muscle protein) will be used directly by the muscle for energy. Finally, during the last minute or so, the runner will make a final effort, using the last of her glucose anaerobically. Until that last effort, the athlete must maintain an intensity that permits an adequate supply of oxygen, maintain body temperature by drinking enough water, and maintain sufficient glucose to supply energy.

CARBOHYDRATE SUPPLEMENTATION (SUPERCOMPENSATION)

When muscle glycogen was identified as the limiting factor for the capacity to exercise at intensities requiring 70% to 85% VO_2 max, dietary manipulation to maximize glycogen stores followed naturally. The most popular subject for research of this nature has been the marathon runner

or cross-country skier, because of the prolonged physical taxation of these events and the fact that the athlete's performance is readily measurable by the time required to complete the course. The major dietary concern to emerge in the endurance training of marathon runners was how to elevate muscle glycogen to above-normal (supercompensated) levels. In sporting vernacular, maximizing glycogen content by dietary manipulation is referred to as "carbohydrate loading." The so-called classical regimen for carbohydrate loading resulted from investigations in the late 1960s by Scandinavian scientists [22]. This regimen involved two sessions of intense exercise to exhaustion to deplete muscle glycogen stores, separated by 2 days of low-carbohydrate diet (<10%) to "starve" the muscle of carbohydrate. This interval was followed by 3 days of high carbohydrate diet (>90%) and rest. The event would be performed on day 7 of the regimen. On completion of this regimen, muscle glycogen levels approached 220 mmol/kg wet weight (expressed as glucose residues), more than double the athlete's resting level. However, because of various undesirable side effects of the classical regimen, such as irritability, dizziness, and a diminished exercise capacity, a less stringent regimen of diet and exercise has evolved that produces comparably high muscle glycogen levels.

In the modified regimen, runners perform "tapered down" exercise sessions over the course of 5 days, followed by 1 day of rest. During this time, 3 days of a 50% carbohydrate diet are followed by 3 days of a 70% carbohydrate diet, generally achieved by consuming large quantities of pasta, rice, or bread. The modified regimen, which can increase muscle glycogen stores 20% to 40% above normal, has been shown to be as effective as the classical approach, with fewer adverse side effects.

Figure 7.13 illustrates graphically the amount of muscle glycogen formed as a result of each regimen. Predictably, the supercompensation of muscle glycogen by either approach has been shown to improve performance in trained runners during races of 30 km and longer. It did not improve performance in shorter races (<21 km), because glycogen depletion is not the limiting factor in such events. Other nutritional factors involving carbohydrate intake may enhance performance, as discussed in the next section.

DIETS FOR EXERCISE

A thorough discussion of all of the factors that influence the choices of food for those involved in some form of strenuous exercise is far beyond the scope of this text. Interested readers should refer to the Suggested Readings on the subject at the end of this chapter. A few broad issues are considered in this section. People that engage in strenuous exercise are extremely diverse in their nutrient needs. A few individuals are world-class athletes who work at getting every small, but meaningful, increase in performance

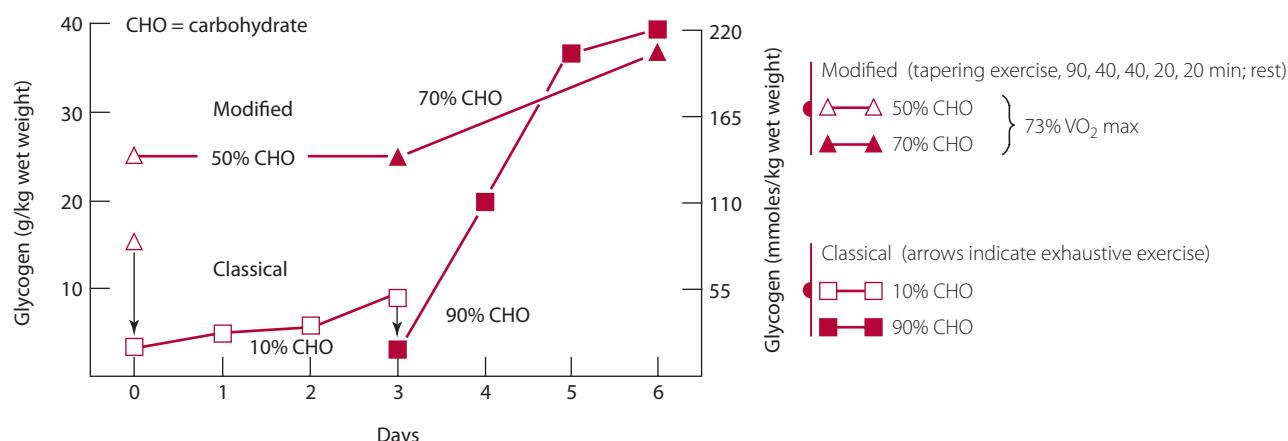


Figure 7.13 Schematic representation of the “classical” and modified regimen of muscle glycogen supercompensation. Source: From Sherman WM. Carbohydrate, muscle glycogen, and muscle glycogen supercompensation. In: *Ergogenic Aids in Sport* by Williams MH, Champaign, IL: Human Kinetics Publishers, 1983, p. 14. Reprinted by permission.

they can, including controlling their nutritional intake. Many more are recreational athletes who would like to follow a similar regime for their sport and their health.

Nutritional needs and, therefore, food selection differs depending on whether one is engaged in an endurance activity or a strength activity. Considerable advice is available about nutrition, diet, and food selection. Some of it is reliable, but much is not, and telling the difference is not always easy.

Macronutrients One of the first considerations in planning a diet for someone involved in strenuous exercise is its macronutrient makeup. The diet must have adequate energy (calories) intake to balance the level of energy expenditure consistent with the desired body composition (see Chapter 8). Endurance athletes tend to focus on carbohydrate. If you are engaged in a long endurance activity, consuming a diet that is high in carbohydrate (>65% of calories) is desirable to maintain elevated muscle glycogen levels. This issue is discussed more fully below. An endurance athlete should not restrict carbohydrate intake. Carbohydrate must be available to supply the four carbon intermediates necessary in the TCA cycle so that β -oxidation of fatty acids can take place.

Strength athletes tend to focus on protein. It does require protein to build and repair muscle and lean body mass. The amount of protein needed for a strength athlete is not fully agreed upon by all recommending groups. The recommended intake for protein range from the RDA of 0.8 gm/kg for more sedentary people to 1.2 to 1.8 gm/kg for strength athletes like body builders and weight lifters (see Chapter 6). Many of these athletes consume much more than the amount proven to provide a benefit in performance.

The recommendations for fat intake for athletes are generally below the intake recommended for the more sedentary population of less than 30% of calories. For athletes in

serious competition in endurance sports, intake of 10% or less of calories are common (but not recommended). High carbohydrate intake is so strongly emphasized that they drop their fat intake to levels below the amount necessary to obtain desired amounts of essential fatty acids. Low-fat diets are generally not very palatable and are difficult to maintain, so most athletes do not stay on these diets long enough to develop essential fatty acid deficiencies.

Meal Frequency The eating patterns of the population are very difficult to define and quantify. Many people in the United States eat three large meals a day, with the largest meal being the evening meal. For athletes, the recommendations are generally to divide the day's food intake into multiple small meals, such as six or so, of equal size. The rationale is that this pattern prevents any sharp spike in blood insulin levels, so the muscle always has a supply of substrate to repair and build muscle and replenish glycogen following exercise. Verifying the value of this recommendation from experimental data is difficult, however.

Pre-event Meal The timing of the final meal before intense exercise is crucial, because fasting reduces the labile glycogen stores of liver. Also, carbohydrate meals consumed too close in time to the event may cause **hyperinsulinemia**. Stimulation of insulin release just before an event results in a rapid reduction in plasma glucose, which significantly impairs work capacity. Exercise permits a rapid uptake of glucose by the muscle in addition to the insulin-stimulated uptake. Elevated plasma insulin also inhibits liver glucose output and the normal rise of plasma free fatty acids. Under such conditions, excessive muscle glycogen degradation occurs, resulting in early fatigue. The final meal before intense exercise should be consumed several hours (3–4 hours) before the event so that the stomach is empty, to avoid stimulating insulin levels and to allow for rapid water absorption. For

long endurance events, the meal generally should be high in complex carbohydrates and low in fat, conditions that promote rapid emptying of the stomach. The nature of the food consumed to meet nutritional objectives is up to the athlete.

An isotonic or hypotonic beverage containing carbohydrate 15 to 20 minutes before the event provides extra dietary glucose without stimulating insulin release. For prolonged events (longer than 90 minutes), consuming fluid containing some carbohydrate helps to maintain fluid balance and blood glucose levels (see Chapter 14). Balance must be maintained to allow the liquid to empty rapidly from the stomach and the carbohydrate to be rapidly absorbed. A full discussion of these factors is beyond the scope of this text. In brief, the beverage should be cool, not cold, be isotonic or hypotonic, and contain glucose or polyglucose. Large amounts of fructose should not be included because of its slow absorption rate.

Glycemic Index The form of carbohydrate ingested is also an important consideration in optimizing endurance performance. The principal factor in this regard is the glycemic index (GI) of the food (see Chapter 3 for a full explanation of glycemic index). Potato starch is considered to have a relatively high GI, though not as high as the simple sugars. Generally, consuming carbohydrate with a low to moderate GI before the performance is preferable to consuming high-GI carbohydrate because the hyperinsulinemic effect of high-GI food, as mentioned earlier, rapidly reduces blood glucose, suppresses release of fatty acids from store, and inhibits hepatic glycogenolysis.

After a prolonged event, however, the reverse is true with respect to GI. Immediately after a glycogen-depleting event, liver and muscle glycogen levels are very low, and glycogen levels recover faster if a high-GI food or beverage is consumed. There is a period following a glycogen-depleting activity when muscle glycogen can be replaced rapidly [23]. The foods consumed can be as simple as wedges of orange or apple or one of the sports drinks containing glucose, sucrose, or polyglucose. Recovery depends on replacing lost body water, rebuilding glycogen levels, rebuilding lost muscle protein, and, for very long events, restoring electrolyte balance. This last topic will be covered in more detail in Chapter 14.

NUTRITIONAL ERGOGENIC AIDS

The word *ergogenic* is derived from the Greek word *ergon*, meaning “work,” and is defined as increasing work or the potential to do work. An ergogenic aid does not have to be nutritional; it can also be mechanical. For example, a running shoe or body suit to improve aerodynamics can be a mechanical ergogenic aid. This discussion is limited to nutritional ergogenic supplements, or ergogenic aids. Often these substances are part of a normal diet, or they

may be cellular metabolites that are ingested in an effort to enhance the capacity for sport, exercise, and physical performance. Several nutritional practices have ergogenic properties that are not necessarily considered ergogenic supplementation, for example, carbohydrate loading and fat loading. Fat loading has been purported to “spare” the more limited carbohydrates. As mentioned previously, fats are the major fuel source for exercise below 50% $\text{VO}_2 \text{ max}$. It is not commonly used.

Nutritional ergogenic supplements must also be distinguished from ergogenic drugs, such as anabolic steroids or stimulants. The risks of using anabolic steroids are so great that they have prompted the enactment and enforcement of laws prohibiting their use. The compulsion for improved performance among athletes has led to an enormous increase in the testing and use of nutritional ergogenic aids. As expected, the literature dealing with the subject has expanded with equal zeal. Many supplements that have not been fully tested for either safety or efficacy have been recommended through the lay press. The information presented here is restricted to the theoretical basis for using them and a brief overview of what is known about the effectiveness of ergogenic supplementation.

A dichotomy appears to exist between the widespread public use of certain supplements and the lack of scientific support for such use. A problem for researchers is the common perception of subjects under study that they simply “feel better” as a result of supplementation, even though actual physiological changes may not be documented by the research. In other words, psychological effects are adding a new dimension to the testing of ergogenic aids. These effects must be considered along with true physiological effects, because as mood and mental outlook improve, so does physical performance—the reason for using supplements in the first place. For all nutritional ergogenic aids, the placebo effect is substantial. The level of athletic performance is influenced by psychological factors. By “believing” that a certain supplement will make you perform better, you may actually perform better. Often a theoretical “rationale” exists for supplement use, but it does not necessarily translate into enhanced performance.

The following section lists micronutrient ergogenic supplements that have been consumed on a broad basis. The supplements chosen for description were selected on the basis of their reputed efficacy from a much longer list of hit-or-miss trial substances. In most instances, research results neither totally support nor totally refute supplement efficacy but instead are divided in their findings. This section occasionally refers to the number of “pro and con” study conclusions to help the reader evaluate a substance’s efficacy. Although specific references are not included, they, along with many more pertinent sources of information, are available to the interested reader [24–26].

Amino Acids

Arginine Arginine in large oral doses has been reported to elicit the release of somatotropin. Somatotropin, which has been called insulin-like growth factor, stimulates protein synthesis. Arginine also has been reported to increase the secretion of growth hormone.

Ornithine Oral doses of ornithine have also been shown to stimulate the release of somatotropin. At the levels required for somatotropin release, however, the side effect of osmotic diarrhea is common. Both arginine and ornithine are purported to be beneficial in resistance training and to increase growth hormone release.

Aspartate Salts The potassium-magnesium salts of aspartate have been marketed as an antifatigue agent. Their use has been questioned, however, and the benefit is more likely a placebo effect. The aspartate salts may have some benefits in endurance events if taken in high doses. Time to exhaustion has been reported to be increased.

Branched-Chain Amino Acids Branched-chain amino acids (isoleucine, leucine, and valine) have been hypothesized to benefit endurance activities by influencing the level of serum tryptophan. BCAAs compete with tryptophan for entry into the brain. One theory on fatigue is that brain tryptophan is converted to serotonin, which causes fatigue. This conversion may be one of several factors that bring about fatigue. BCAAs are also used by muscle for energy near the end of very long endurance events. It has been suggested that consuming BCAAs before an event provides energy toward the end of the event and thereby reduces the amount of muscle breakdown.

Antioxidants

Endurance exercise increases the amount of oxygen moving into the muscle. Increased exposure to large volumes of oxygen in turn increases the generation of free radicals, which are involved in fatigue and damage to the muscle cell membrane. This information provides the rationale for using antioxidants to prevent muscle damage and delay fatigue. Many antioxidants have been used, including vitamin C, vitamin E, and selenium. Coenzyme Q₁₀ also has antioxidant activity, though its use as an ergogenic aid is based on other properties.

Herbs

Much interest has recently been directed toward herbal preparations. Evaluating and comparing studies of these preparations is difficult, because the way herbs are collected, processed, and grown influences the active components. One class of herb, ephedra, was previously used for its ephedrine content. The risk of harmful side effects or death has discouraged its use and caused it to be banned in most

sports. The FDA banned the sale of ephedra-containing supplements in 2004. The ban was later removed after the FDA lost a court challenge.

The Ginsengs The most widely used and studied herbs are the ginsengs. Some purported ergogenic benefits of *Panax* (Chinese/Korean) *ginseng* include:

- increased run time to exhaustion (three out of seven studies)
- increased muscle strength (one out of two studies)
- improved recovery from exercise (three out of four studies)
- improved oxygen metabolism during exercise (seven out of nine studies)
- reduced exercise-induced lactate (five out of nine studies)
- improved auditory and visual reaction times (six out of seven studies)
- improved vitality and feelings of well-being (six out of nine studies)

These benefits have most consistently been reported following supplementation over more than 8 weeks [27].

Caffeine

Ergogenic effects of caffeine are seen in endurance events. The greatest effect is seen in people who do not consume caffeine on a regular basis. Caffeine is a CNS stimulant that increases blood flow to the kidneys (thus acting as a diuretic) and stimulates the release of fatty acids from adipose. Sport regulatory bodies have changed their position on caffeine use several times. It was banned for a period before 1972 and then removed from the banned list. Regulators then set an upper limit for its use. Caffeine was removed from the banned list of stimulants before the 2004 Olympics. The use of caffeine is now being reconsidered once again.

Intermediary Metabolites

Bicarbonate Bicarbonate is a primary buffering agent in the body. Athletes competing in short anaerobic events (lasting only a few minutes) build up lactic acid. The lowering of blood pH is one factor that leads to fatigue. Theoretically, loading with sodium bicarbonate would delay the drop in pH and thereby delay fatigue. Studies have supported this benefit, and it is often mentioned in reviews of ergogenic aids. In conversations with many sprint athletes and coaches, however, none reported having used sodium bicarbonate, nor did they know of anyone who did.

Carnitine L-carnitine is used by the body to transfer acyl CoA from the cytoplasm of a cell into the mitochondria. This is the theoretical basis for the use of carnitine as a

nutritional ergogenic aid. In people fed parenterally for long periods of time, fatty acid use can be enhanced by supplementation with carnitine. People with chronic cardiovascular disease have also been shown to benefit from carnitine. For the athlete, studies that show benefit and those that do not are about even in number.

Coenzyme Q₁₀ The theoretical basis for coenzyme Q₁₀ as an ergogenic aid stems from its pivotal role in electron transport and production of ATP in the mitochondrion. Clinical studies have shown its safety and use in cardiovascular disease. Supplementation with coenzyme Q₁₀ longer than 4 weeks has been purported to provide benefits for the long-term endurance athlete. This benefit has not been shown conclusively, however.

Creatine Muscle creatine is part of the ATP-CP energy system that supplies the initial energy during the first few seconds to minutes of exercise. The theoretical basis for using creatine as a nutritional ergogenic aid is that saturating muscle with creatine increases the amount of creatine phosphate in the muscle. Creatine is effective for short, intense exercise. However, taking it is associated

with some risk. People taking creatine appear to add 1 to 2 kg of water weight. Those taking creatine in hot, humid environments have become dehydrated and more susceptible to heat stress. Deaths have been reported.

Other Many other nutritional materials have been recommended in the lay literature as possessing ergogenic properties, including minerals such as calcium, magnesium, zinc, iron, phosphates, chromium, boron, vanadium, and most vitamins. Reviews of mineral supplements [16] suggest that performance enhancement is not well established and that the major benefit of mineral supplementation lies in the correction of deficiencies, should they exist.

General problems of research design remain as the popularity of nutritional ergogenic supplements surges forward. Many ergogenic effects may be attributed to mental and psychological changes, and it behooves future researchers to rule out these effects to establish strictly physiological effects. The fact that the number of studies finding “for” performance enhancement is nearly equaled by the number of those finding “against” enhancement testifies to the difficulty involved in researching this important field.

SUMMARY

Animal survival depends on a constant internal environment maintained through specific control mechanisms. Controls, operative at all levels (cellular, organ, and system), integrate energy metabolism and allow the body to adapt to a wide variety of environmental conditions. Primary among the mechanisms of adaptation is the regulation of metabolism through the cooperative input of the nervous, endocrine, and vascular systems. In the normal operation of these systems, metabolic pathways may be stimulated, maintained, or inhibited, depending on the conditions imposed on the body. A pointed example of metabolic adaptation is the shift that occurs in substrate use and metabolic pathways in answer to changes in the body's nourishment status (i.e., fed, fasting, and starvation states).

Metabolic syndrome is an example of the interrelation of nutrient intake and metabolism. This syndrome is

a clustering of risk factors for cardiovascular disease, chronic kidney disease, and type 2 diabetes.

The physical stress of exercise and sports presents an interesting challenge to the regulatory capacity of the body to provide the additional energy needed by exercising muscles. Substrates fueling this energy include plasma free fatty acids, plasma glucose, muscle glycogen, and muscle triacylglycerols, and their use varies according to the intensity and duration of the exercise. Many substances have been tested for their ergogenic properties in attempts to improve performance and muscle triacylglycerols, and their use varies according to the intensity and duration of the exercise. In most cases, test results remain controversial, and more research is needed to establish which of the reputed ergogenic aids produce true physiological improvement.

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Web Sites

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National Agricultural Library at USDA. Then click on Food and Nutrition.
- www.umass.edu/cnshp/index.html
Center for Nutrition in Sport and Human Performance at the University of Massachusetts
- www.cdc.gov/nccdphp/dnpa
Center for Disease Control and Prevention Division of Nutrition and Physical Activity.
- www.ajaxn.org
American Journal of Clinical Nutrition
- www.gssiweb.com
Gatorade Sports Science Institute
- www.beverageinstitute.org
The Beverage Institute for Health and Wellness

Diabetes: Metabolism Out of Control

Diabetes mellitus, the disease characterized by the body's inability to metabolize glucose, manifests as one of two types: type 1, formerly called insulin-dependent diabetes mellitus (IDDM); and type 2, formerly called non-insulin-dependent diabetes mellitus (NIDDM). The long-term consequences of diabetes demonstrate that lipid metabolism is also involved. The two types of diabetes result from very different mechanisms and are discussed separately. Current theories on the etiology and characteristics of these two classifications of diabetes are shown in Figure 1.

Type 2 Diabetes

Type 2 diabetes accounts for 80% to 90% of all reported cases of the disease. The cause of type 2 diabetes has not been completely resolved, but it appears to be associated with insulin resistance in adipose tissue and muscle. This condition is caused not by a failure of target cells to bind insulin but by a postbinding abnormality, arising somewhere in the sequence of events that follows the binding of insulin to its receptor and leads to the cell's normal response to that signal. Experimental evidence suggests that a primary cause

for the interrupted insulin signal may be compromised synthesis or mobilization of the cell's glucose transporters (refer to the section "Glucose Transporters," Chapter 3).

In skeletal muscle cells, insulin resistance associated with type 2 diabetes is caused by a reduction in glucose transporter activity, specifically the failure of the vesicles to translocate in response to insulin (Figure 3.10). The error can be thought of as a block or short-circuit in the insulin signal that normally initiates the translocation process. The result is a reduced concentration of transporters at the cell surface and a consequent reduction in the rate of glucose uptake. Although a similar defect was found in adipocytes of type 2 diabetic patients, it is not the major cause of the insulin resistance in these cells. Rather, the consequence of type 2 diabetes in adipocytes is a marked depletion of mRNA encoding the GLUT4 transporter, resulting in depleted intracellular stores of the protein [1]. This defect is pretranslational, meaning that it interferes with protein synthesis at a level before the translation process, the step that requires mRNA as template. Therefore, even if the vesicle translocation process

were not compromised, an inadequate number of surface receptors would still be expressed upon insulin stimulation.

Insulin resistance has also been described in obesity as well as in type 2 diabetes. Insulin resistance in obesity is mechanistically similar to the type 2 diabetes effect on adipocytes. Reduction in GLUT4 mRNA in obese subjects results in a decrease in de novo synthesis of the transporter. Furthermore, the extent to which mRNA expression is suppressed appears to relate directly to increasing adiposity.

In summary, type 2 diabetes is characterized by insulin resistance in peripheral target tissues because of a diminished population of functional glucose transporters. In muscle cells, the defect appears to arise from a failure, on insulin stimulation, of vesicle-bound transporters to translocate to the plasma membrane. In adipocytes, translocation is also compromised, but the major mechanism for insulin resistance in these cells, in both type 2 diabetes and obesity, is a pretranslational depletion of GLUT4 mRNA. In the latter stages of type 2 diabetes, the pancreas loses its ability to produce insulin. Insulin therapy is more likely to be used at this stage.

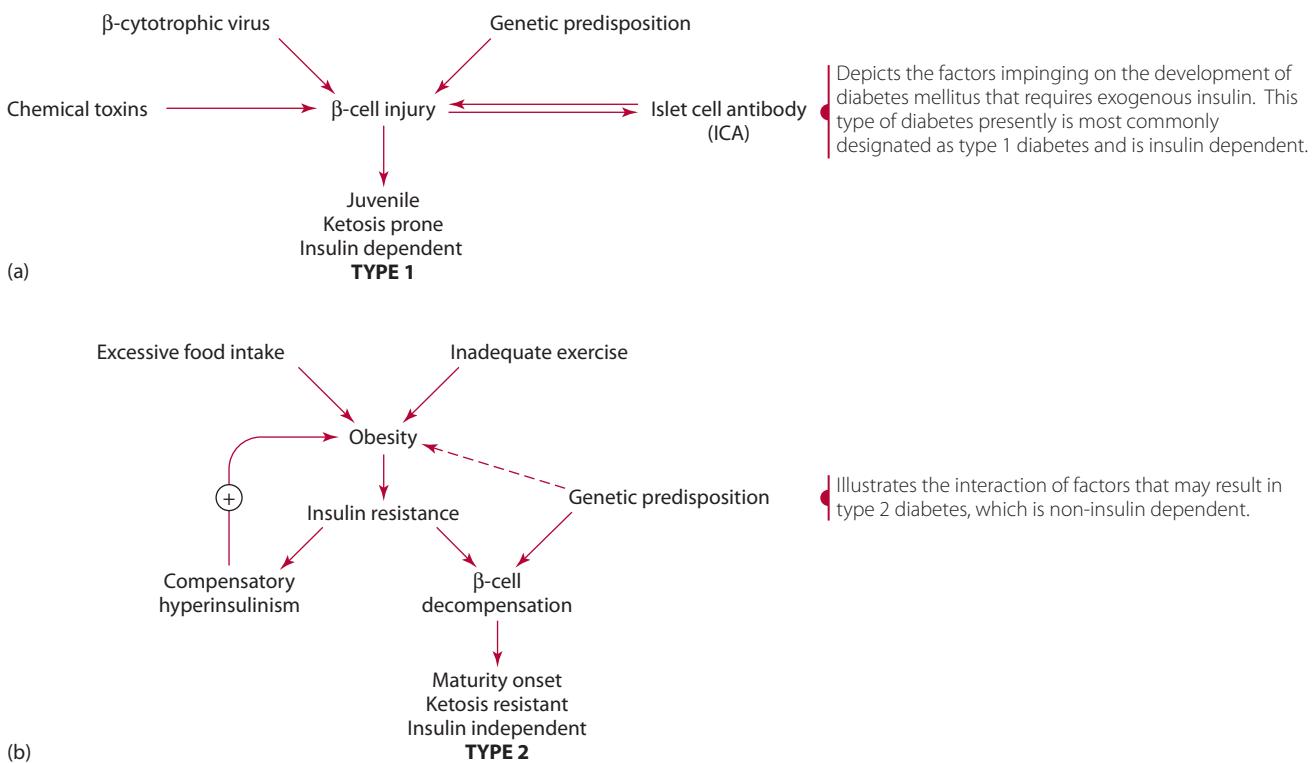


Figure 1 Overview of present theories of diabetes mellitus etiology.

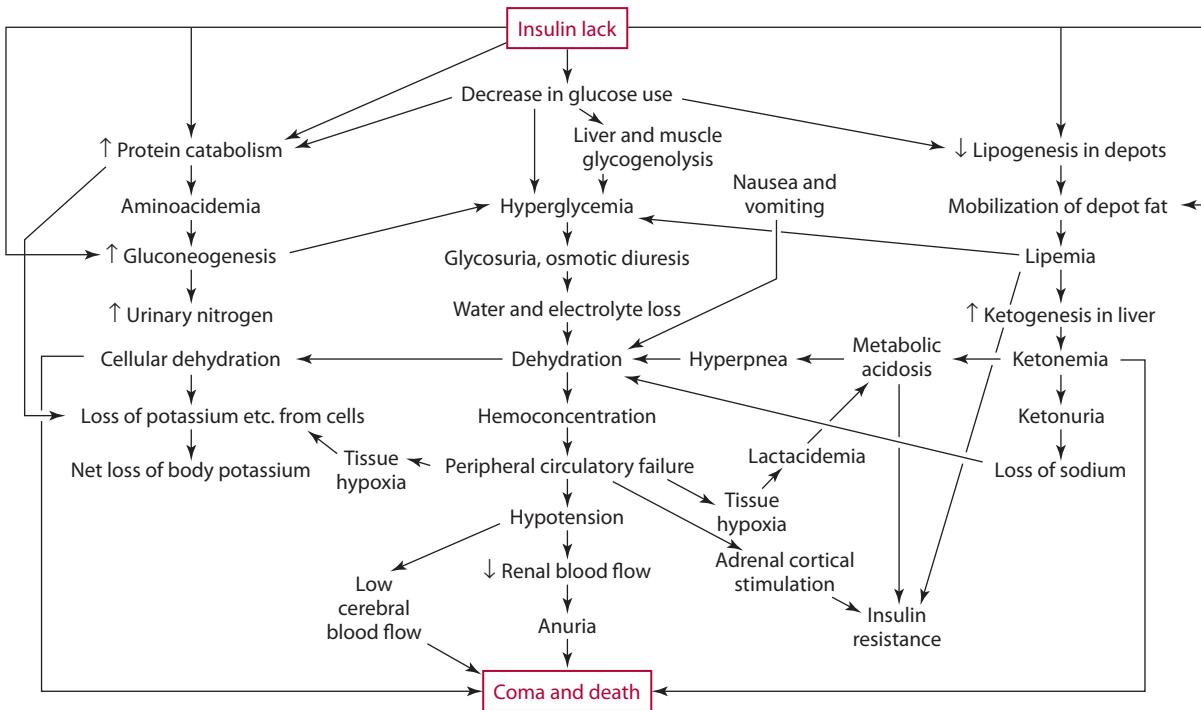


Figure 2 Composite summary of pathophysiology of diabetic acidosis. Particularly striking are the aberrations in the metabolism of carbohydrates, lipids, and protein caused by an insulin lack and the interconnections among these altered metabolic pathways. Source: Tepperman D, Tepperman H. *Metabolic and Endocrine Physiology*, 5th ed. Chicago:Year Book, 1987, p. 284.

Type 1 Diabetes

The hyperglycemia of type 1 diabetes can be attributed to a primary failure of the β -cells of the pancreas to produce and secrete insulin. Type 1 diabetes is regarded as an autoimmune disease in which the pancreatic islet cells, which are composed largely of β -cells, become targets of an immune response. This assault ultimately causes cellular dysfunction of the β -cells, which become unable to produce insulin. Factors that trigger the immune attack remain unknown.

Figure 2 emphasizes the crucial role of insulin in regulating metabolism and the metabolic events set in motion by lack of insulin. An absence of insulin not only inhibits the use of glucose by muscles and adipose tissue but also sets in motion a sequence of events that, without effective intervention, results in coma and death of the affected person. Insulin acts on metabolism in various ways, most of which have the effect of lowering blood glucose. These actions include decreasing hepatic glucose output while increasing glucose oxidation, glycogen deposition, lipogenesis, protein synthesis, and cell replication. In the absence of insulin, all the hormones favoring catabolism and the raising of blood glucose operate without opposition. The direction of metabolism in response to these catabolic hormones is shown in Figure 7.9, which depicts the body's adaptation to fasting. In diabetes, however, the responses are much more violent than those that occur

in the body's adaptation to fasting or starvation. In starvation, the purpose is to maintain a blood glucose level sufficient to meet the crucial demands of the CNS and RBCs. The unrestrained action of the catabolic hormones in the absence of insulin, along with the dramatically decreased use of glucose caused by lack of insulin, results in aberrations in metabolism. Not only is metabolism of carbohydrate, fat, and protein affected, but water and electrolyte imbalance also occurs.

Hyperglycemia, the hallmark of diabetes, is caused by decreased glucose uptake by the cells of muscle and adipose and increased hepatic glucose output and results in an osmotic diuresis that proves fatal if uninterrupted (Figure 2). The water and electrolytes lost through this diuresis lead to dehydration compounded by increased insensible water loss during the **hyperpnea** (abnormally rapid breathing) of metabolic acidosis. Metabolic acidosis results from the excessive ketogenesis occurring in the liver.

Peripheral circulatory failure, a consequence of severe hemoconcentration, leads to tissue hypoxia with a consequent shift of the tissues to anaerobic metabolism. Anaerobic metabolism raises the concentration of lactic acid in the blood, thereby worsening the metabolic acidosis.

The ketonuria along with glucosuria associated with acidosis causes an excessive loss of sodium from the body, and loss of this extracellular cation further compromises body water balance. A net

loss of potassium, the chief intracellular cation, accompanies increased protein catabolism and cellular dehydration, both of which characterize uncontrolled diabetes.

The normal flow of substrates following food intake, as depicted in Figure 7.7, depends largely on the secretion of insulin. Insulin exerts a potent, positive effect on anabolism, emphasized in the figure, while inhibiting catabolic pathways. Figure 2, in contrast, shows metabolism out of control when the inhibiting effect of insulin is lacking and conservation of energy is impossible. Diabetes is a vivid negative example of the integration of metabolism and the importance of metabolic regulation (homeostasis) to continuance of life.

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Web Sites

www.diabetes.org

American Diabetes Association

www.idfcure.org

Juvenile Diabetes Foundation

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8

Body Composition, Energy Expenditure, and Energy Balance

Body Weight: What Should We Weigh?

Body Mass Index

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Infrared Interactance

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Positive Energy Balance

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PERSPECTIVE

Eating Disorders

Body weight and composition are an important area in the study of nutrition. The current rapid increase in the prevalence of obesity in this country is making headlines. Government, medical, public health, and nutritional professionals are examining the etiology and developing strategies to stop or reverse this trend of increased obesity. This chapter explores what we should weigh, our body composition, and how to determine the proportions of fat and lean body mass. The chapter also addresses the balance between energy intake and expenditure as well as the impact on our weight and body composition when energy is not in balance. Understanding the influences of genetics and hormones that regulate our appetite, weight, and body composition will assist in developing and implementing interventions.

Body Weight: What Should We Weigh?

Recognition of body weight as an indicator of health status is probably universal and as old as humanity itself. In fact, in 1846 English surgeon John Hutchinson published a height-weight table based on a sample of 30-year-old Englishmen and urged that future census taking include such information, which he believed to be valuable in promoting health and detecting disease [1]. Today, scientists and health professionals recognize that the risk of many diseases—including heart disease, stroke, diabetes mellitus, hypertension, osteoarthritis, infertility, and some cancers (endometrial, colon, and kidney)—increases with excess body fat. Because body fat is so difficult to measure, body weight is a good proxy in the nonathletic population. Furthermore, a low body weight may indicate malnutrition or an eating disorder and may pose risks for other diseases, such as osteoporosis. What represents too much weight or too little weight for a given height? Unfortunately, recommendations from health experts vary. This chapter covers some of the currently accepted approaches to weight assessment.

BODY MASS INDEX

Body mass index (BMI), first described in the 1860s and known as Quetelet's Index, is at present one of the most accepted approaches to assessing appropriate weight for a given height. The body mass index is considered to indicate body adiposity but does not measure body fat. Body mass index is calculated from a person's height and weight as shown in this formula:

$$\text{Body mass index} = \frac{\text{Weight}}{\text{Height}^2}$$

with weight measured in kilograms (kg) and height measured in meters (m) and raised to a power of 2.

Body mass index is considered a good index of total body fat in both men and women and has generally replaced calculations of percent relative body weight and percent ideal body weight (see the section “Formulas”) for classifying people as underweight or overweight. For adults, classification of weight based on body mass index by the National Institutes of Health [2–4] is as shown in Figure 1.

- BMI $<18.5 \text{ kg/m}^2$, underweight (with <16 suggesting a possible eating disorder)
- BMI 18.5 to 24.9 kg/m^2 , healthy/low health risks
- BMI 25–29.9 kg/m^2 , overweight and associated with increased risk of disease
- BMI 30–34.9 kg/m^2 , obese (class I) and associated with further increased risk of disease
- BMI 35–39.9 kg/m^2 , obese (class II) and associated with higher risk of disease
- BMI $\geq 40 \text{ kg/m}^2$, extremely or morbidly obese (class III)

As an example of how BMI changes with age in children, the body mass index growth curve for boys and girls 2 to 20 years of age is shown in Figure 8.2. Underweight is $<5\text{th}$ percentile, a healthy weight is between the 5th and 85th percentile, at risk of being overweight is between 85th and 95th percentiles, and $>95\text{th}$ percentile is considered being overweight.

Using the formula to calculate the BMI of a 5-foot 11-inch (or 71-in) man weighing 165 pounds would involve two conversions before plugging numbers into the body mass index formula. First, to convert weight in pounds (lb) to weight in kilograms (kg), divide by 2.2 (because there are 2.2 lb per 1 kg): $165 \text{ lb} \div 2.2 \text{ lb/kg} = 75 \text{ kg}$. Next, convert height in feet and inches to meters (m). Because there are 39.37 in/m, divide the man’s height of 71 in by 39.37 in/m to get 1.803 m. With weight in kilograms and height in meters, the formula can be used: $\text{BMI} = 75 \text{ kg} \div (1.803 \text{ m})^2 = 75 \text{ kg} \div 3.25 \text{ m}^2 = 23.1 \text{ kg/m}^2$.

Although the body mass index is a valuable tool for assessing weight, like many other methods it does not determine body composition. Thus, people such as athletes may have large amounts of lean body mass and a high body mass index (and thus be considered overweight or obese by classification) but have a low percentage of body fat.

FORMULAS

In addition to the body mass index other formulas are available to calculate ideal body weight (IBW). One of the more popular formulas is that by Devine [5,6]:

$$\text{IBW for men} = 50 \text{ kg} + 2.3 \text{ kg/in} > 5 \text{ ft}$$

$$\text{IBW for women} = 45 \text{ kg} + 2.3 \text{ kg/in} > 5 \text{ ft}$$

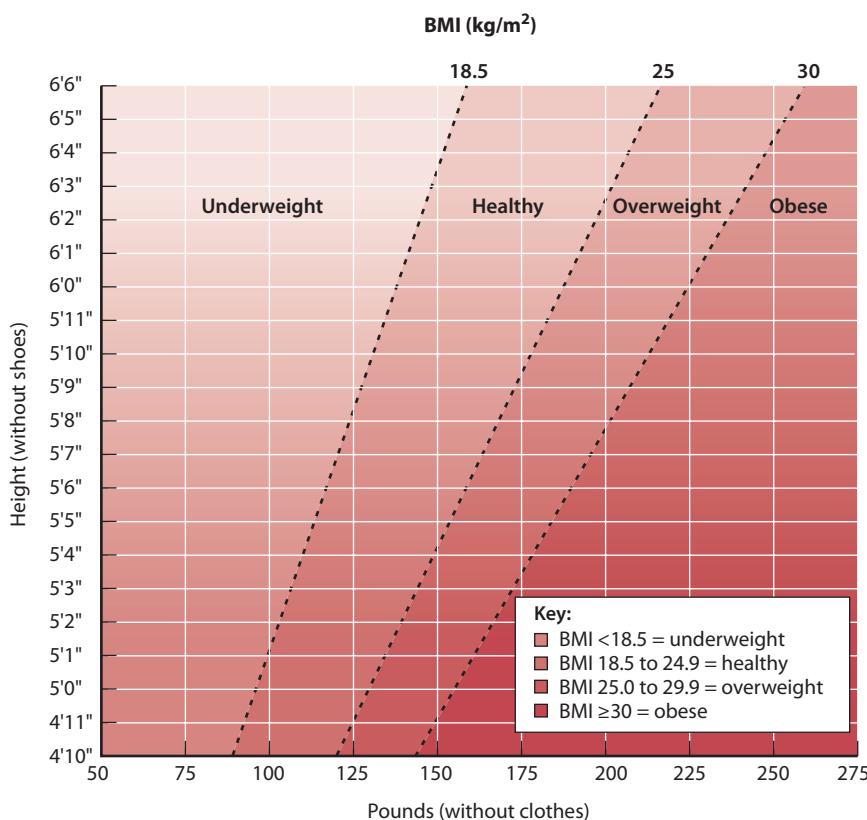


Figure 8.1 BMI values used to assess weight.

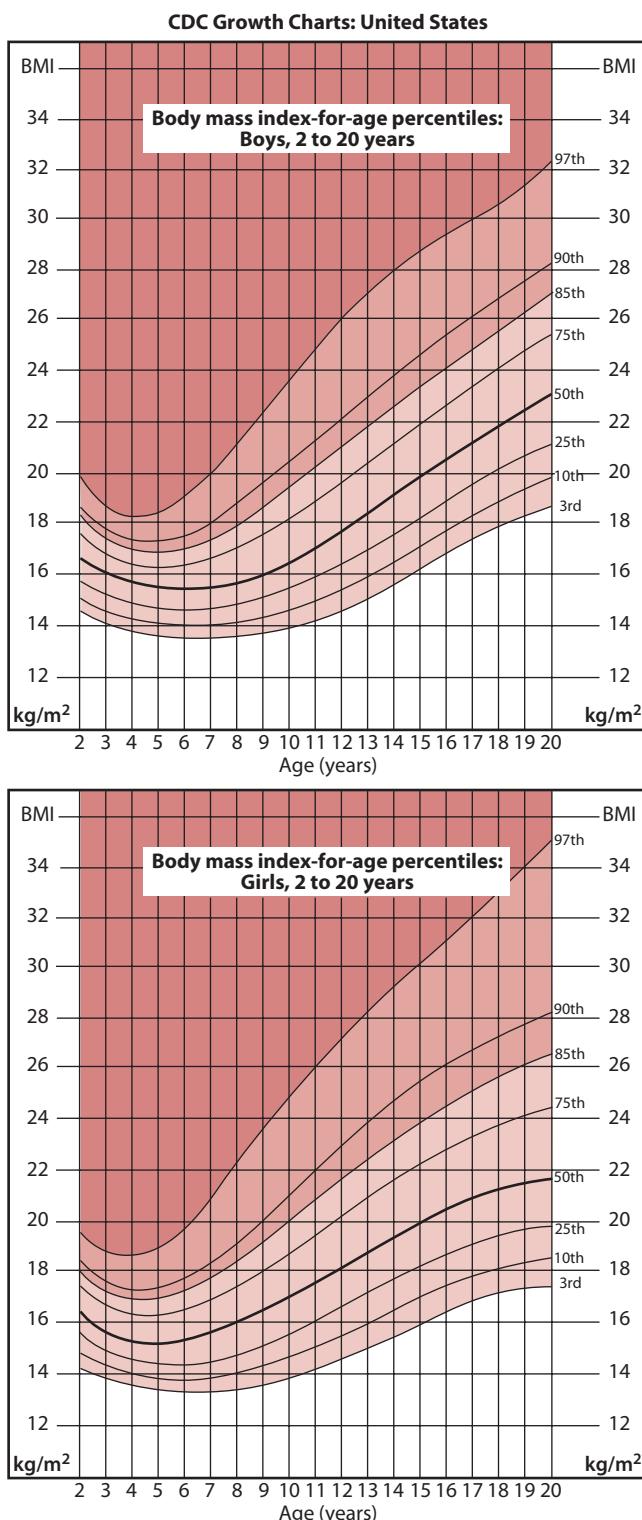


Figure 8.2 Example of growth curves (2 to 20 years): boys' and girls' body mass index-for-age-percentiles.

These formulas have been modified somewhat and converted into the following familiar empirical formulas for those with a medium-size body frame:

$$\text{IBW for men} = 110 \text{ lb} + 5 \text{ lb/in} > 5 \text{ ft}$$

$$\text{IBW for women} = 100 \text{ lb} + 5 \text{ lb/in} > 5 \text{ ft}$$

A slightly modified formula for men is also used:

$$\text{IBW for men} = 106 \text{ lb} + 6 \text{ lb/in} > 5 \text{ ft}$$

A 10% range minus or plus the calculated ideal weight (which allows for differences in weight from a small or a large frame, respectively) is also usually included in the Devine formula.

Frame size traditionally is assessed using either of two methods based on measurements of wrist or elbow breadth and height. The method of determining the elbow breadth is shown in Figure 8.3. In addition, the frame index 2 considers not only elbow breadth and height but also age and gender. The frame index 2 value is determined by the following formula:

$$\text{Frame index 2 value} = \frac{\text{Elbow breadth}}{\text{Height}} \times 100$$

with elbow breadth measured in millimeters (mm) and height measured in centimeters (cm) [7]. Once calculated, a person's frame index 2 value is compared with age-based values (shown in Table 8.1) to determine small, medium, or large frame size.

Using the formulas, a man who is 5 ft, 11 in tall with a medium frame should weigh either 165 lb ($110 + [5 \times 11] = 165$ lb) or 172 lb ($106 + [6 \times 11] = 172$ lb), depending on which formula is used. If the man has a small frame, ideal body weight would be 10% less than that calculated for a medium frame size (i.e., $165 \text{ lb} - 16.5 \text{ lb} = 148.5 \text{ lb}$ or $172 \text{ lb} - 17.2 \text{ lb} = 154.8 \text{ lb}$, respectively). A female who is 5 ft 6 in tall and has a large frame has an ideal body weight of 143 lb ($100 + [5 \times 6] = 130 \text{ lb} + 13 \text{ lb}$ [accounting for the 10% addition for the large frame size] = 143 lb).

Dividing a person's actual body weight by the ideal body weight calculated (from the Devine formula) for his or her

Table 8.1 Classification of Frame Size Based on Frame Index 2 Values

Age (yrs)	Male			Female		
	Small	Medium	Large	Small	Medium	Large
18.0–24.9	<38.4	38.4 to 41.6	>41.6	<35.2	35.2 to 38.6	>38.6
25.0–29.9	<38.6	38.6 to 41.8	>41.8	<35.7	35.7 to 38.7	>38.7
30.0–34.9	<38.6	38.6 to 42.1	>42.1	<35.7	35.7 to 39.0	>39.0
35.0–39.9	<39.1	39.1 to 42.4	>42.4	<36.2	36.2 to 39.8	>39.8
40.0–44.9	<39.3	39.3 to 42.5	>42.5	<36.7	36.7 to 40.2	>40.2
45.0–49.9	<39.6	39.6 to 43.0	>43.0	<37.2	37.2 to 40.7	>40.7
50.0–54.9	<39.9	39.9 to 43.3	>43.3	<37.2	37.2 to 41.6	>41.6
55.0–59.9	<40.2	40.2 to 43.8	>43.8	<37.8	37.8 to 41.9	>41.9
60.0–64.9	<40.2	40.2 to 43.6	>43.6	<38.2	38.2 to 41.8	>41.8
65.0–69.9	<40.2	40.2 to 43.6	>43.6	<38.2	38.2 to 41.8	>41.8
70.0–74.9	<40.2	40.2 to 43.6	>43.6	<38.2	38.2 to 41.8	>41.8

Source: This table was published in Matarese, L., Gottschlich, M., *Contemporary Nutrition Support Practice*, p. 37. Copyright Elsevier, 2003.

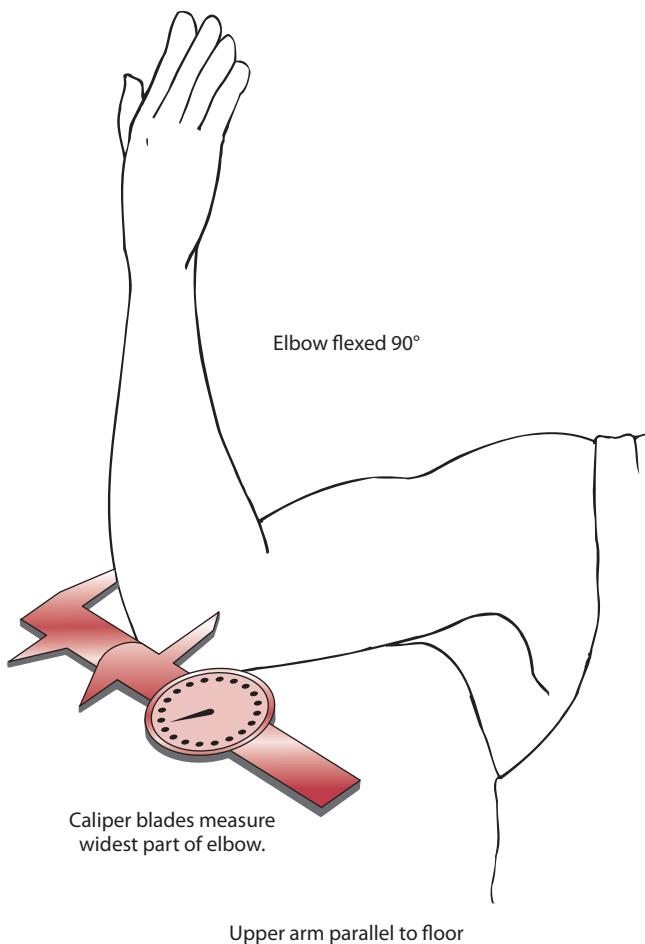


Figure 8.3 Measurement of elbow breadth. Extend your arm and bend the forearm upward at a 90° angle. Keep your fingers straight, and turn the inside of your wrist toward your body. Place the calipers on the two prominent bones on either side of the elbow. Measure the space between the bones with the caliper to the nearest 0.1 cm or 1/8 inch.

estimated frame size gives a value called percentage ideal body weight, as shown:

$$\% \text{ IBW} = \frac{\text{Actual Body Weight}}{\text{Ideal Body Weight}} \times 100$$

Although largely replaced by use of the body mass index, calculation of percent IBW is another approach used for classification of people as overweight, obese, or underweight. For example, people whose body weight is 10% or more below the ideal for a given height (i.e., $\leq 90\%$ IBW) are considered underweight, whereas those whose body weight is 10% or more above the ideal for a given height (i.e., $\geq 110\%$ IBW) are considered overweight. People with a weight for height 20% or more above the ideal (i.e., $\geq 120\%$ IBW) are considered obese.

Using the formula, a male with a medium frame who is 6 ft 3 in tall and weighs 260 lb would have a % IBW of $260 \text{ lb}/(106 \text{ lb} + [6 \text{ lb/in} + 15 \text{ in}]) \times 100$, or $260 \text{ lb}/196 \text{ lb} \times 100$ or 132.7%. At 132.7% IBW, this male would be considered obese.

Regression equations for estimating IBW also have been developed based on the 1959 Metropolitan Life Insurance Company height-weight tables [1] and the ideal body weight tables used by Grant [8] in nutrition assessment. The regression equation is still used even though the Metropolitan Life Insurance Company Tables have been replaced by the use of BMI. The equation based on the 1959 Metropolitan Life Insurance Company tables [9] (with indoor clothing and shoes) is:

$$\text{IBW (lb)} = -139.17 + 3.86(\text{height}) + 9.52(\text{frame}) + 5.01(\text{sex})$$

Based on Grant's tables, corrected for height and nude weight, the equation becomes:

$$\text{IBW} = -133.99 + 3.86(\text{height}) + 9.52(\text{frame}) + 3.08(\text{sex})$$

In these two equations, height is in inches. Values used for frame size are 1 for small, 2 for medium, and 3 for large. Values for sex are +1 for male and -1 for female.

Although measuring both height and weight is relatively easy and can serve as a screen for underweight, overweight, or obesity, no method (weight for height from tables, formulas, or body mass index) is necessarily a valid indicator of the degree of body fatness. The inadequacy of weight as a valid measure of fatness became clear in World War II when A. R. Behnke, a Navy physician, was able to demonstrate by hydrostatic weighing that several football players who had been found unfit for military service because of excessive weight actually had less body fat than controls of normal weight [10]. The excessive weight of these athletes was from hypertrophy of muscles rather than excessive adipose tissue [10]. Behnke's work rekindled interest in studying the composition of the human body, an interest that had lain dormant for about 50 years.

The Composition of the Human Body

The chemical composition of the human body was first described in 1859 in a book that dealt with the chemical composition of food [11]. Analytic chemistry was a rapidly growing science at the time, and figures describing the chemical composition of the different body tissues were compared with those of various foods. Additional chemical composition data from whole-body analysis of fetuses, children, and adults collected during the next few decades represent a direct (rather than indirect) measure of body composition [12–17].

The concept of the reference man and woman was developed in the 1970s [16]. These reference figures provide information on body composition based on average physical dimensions and provide a frame of reference for comparisons. The reference man and woman and the change in

body composition and development with age is provided in Chapter 6 and in Tables 6.8–6.12. In this chapter we focus on the fat mass and fat-free mass and the methods of measuring them. Recall (Table 6.8) that the reference man has 3% essential fat, 12% storage fat (for a total of 15% body fat), 44.8% muscle, 14.9% bone, and 25.3% other components. The reference woman has 12% essential fat, 15% storage fat (for a total of 27% body fat), 36% muscle, 12% bone, and 25% other components. Essential fat includes the fat that is associated with bone marrow, the central nervous system, internal organs, and the cell membranes. The essential fat in females also includes the fat in mammary glands and the pelvic region. Evaluation of body composition has to consider these gender differences. A common way to compare and evaluate the body composition is to consider only two compartments, fat body mass and lean body mass. Lean body mass includes muscle, bone, and the remainder of the body weight. Fat mass is mostly triacylglycerides and other lipid components, with relatively small amounts of water or electrolytes. Lean body mass is much more diverse. It is made up of muscle, bones, and the intra- and extracellular fluids. The differences in the composition of the two compartments are the basis for many of the methods determining body composition. These differences include differences in density (weight for a given volume), the ability to conduct an electrical current, the electrolyte content, and the X-ray density.

Methods for Measuring Body Composition

Division of the body into components is used extensively for *in vivo* studies of body composition. The body can be categorized atomically in terms of its elements—primarily carbon, oxygen, hydrogen, and nitrogen, which make up about 95% of body mass, along with about another 50 or so elements that make up the remaining 5%. Alternately, the body may be thought of from a nutritional or molecular perspective as consisting of water, protein, fat, carbohydrate, and minerals. Multicompartment models to assess body composition rely on calculations of these different nutrient components.

Densitometry, one standard against which other indirect measurements of body composition are evaluated, separates the body into two components: fat mass and fat-free mass [11,15,17]. According to the two-component model of body composition assessment, fat mass includes essential and nonessential fat (triacylglycerides, also called triglycerides), whereas fat-free mass includes protein, water, carbohydrate (glycogen), and minerals [11,15]. The term *lean body mass* is used synonymously with *fat-free* mass but also includes essential body fat [11,17]. Several

methods of body composition assessment are available, some of which are based on the two-component model. Commonly available methods are indirect and provide a means to calculate body components (direct measurement is accomplished only on cadavers). Although different procedures are available, accuracy varies not only with the equipment or method used but also with the technician. Several indirect methods of body composition assessment are reviewed in this section.

ANTHROPOMETRY

Anthropometry estimates body composition through measurement at various circumference and skin fold (fat) sites. Skin varies in thickness from 0.5 mm to 2 mm [18]; thus, fat beneath the skin typically represents most of the skin fold measurement. The assumption is that a direct relationship exists between total body fat and fat deposited in depots just beneath the skin (i.e., subcutaneous fat). Skin fold measurements can be used in one of two ways:

- Scores from the various measurements can be added and the sum used to indicate the relative degree of fatness among subjects.
- Scores can be plugged into various mathematical regression equations developed to predict body density or to calculate percentage of body fat [17,19].

Five sites commonly used for measuring skin fold thickness, shown in Figure 8.4 (labeled A–E), are as follows:

- A. Back of the upper arm (triceps)—a vertical fold is measured at the midline of the upper arm halfway between the tip of the shoulder and the tip of the elbow.
- B. Subscapula—an oblique fat fold is measured just below the tip (interior angle) of the scapula.
- C. Suprailiac—a slightly oblique fold is measured just above the hipbone, with the fold lifted to follow the natural diagonal line at this point.
- D. Abdomen—a vertical fold is measured 1 inch to the right of the umbilicus.
- E. Thigh—a vertical fold is measured at the midpoint of the thigh, between the kneecap and the hip (inguinal crease) [20,21].

Additional sites often include the pectoral (chest), midaxillary, and calf. The right side of the body is used for most measurements if comparisons are being made to standards derived from data from U.S. surveys, because such surveys typically measured the right side of subjects. The handedness of the subject affects skin fold measurements taken on the arm such that measurements on the right exceed those on the left by 0.2 to 0.3 standard deviation units [22].

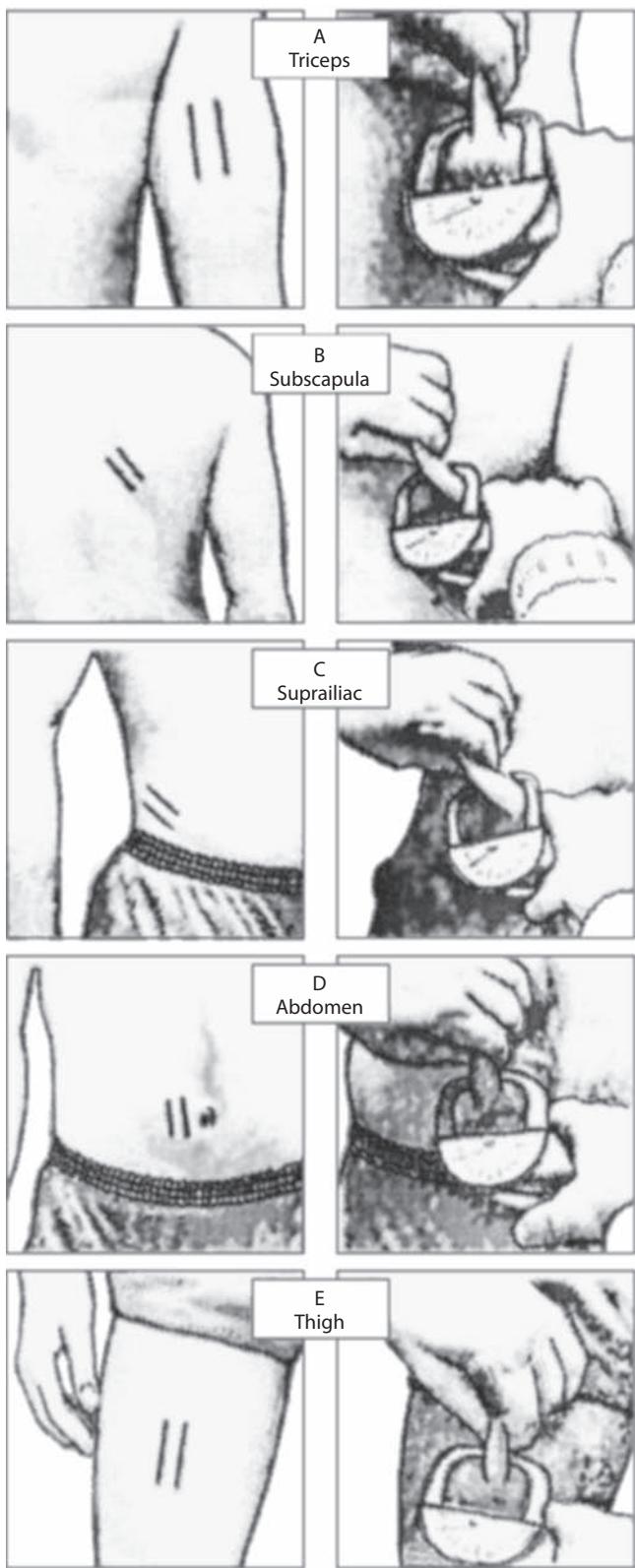


Figure 8.4 Anatomic location of the five fat fold sites: (A) triceps, (B) subscapula, (C) suprailiac, (D) abdomen, (E) thigh.

Bias associated with the side of the measurement, however, is less than error caused by measurement [22]. All measurements should be repeated at least two or three times, and the average should be used as the skin fold value.

Measurement procedures and the use of formulas (see the next paragraph) contribute to procedure error. The precision of skin fold thickness measurements depends on the skill of the anthropometrist; in general, a precision of within 5% can be obtained by a well-trained and experienced anthropometrist [23]. The use of anthropometry for predicting visceral fat content offers limited accuracy [24]. Nevertheless, the method is quite inexpensive compared with other techniques.

Several different equations that are population specific have been developed for calculating total body fat from skin fold sites. Equations developed by Katch and McArdle [20] for predicting total body fat in young (age 17–26 years) men and women from the triceps and subscapular skin folds are shown:

$$\begin{aligned} \text{Young women: percent body fat} = & 0.55(A) + 0.31(B) \\ & + 6.13 \end{aligned}$$

$$\begin{aligned} \text{Young men: percent body fat} = & 0.43(A) + 0.58(B) \\ & + 1.47 \end{aligned}$$

where A = triceps fat fold measured in millimeters and
B = subscapular fat fold measured in millimeters.

Measurements from multiple (at least three) sites are deemed better for overall subcutaneous fat assessment than measurements from only one or two sites [20].

Circumference or girth measurements also may be used to assess body fat. Typical sites of measurement include the abdomen, buttocks, right thigh, and right upper arm. As with skin fold measurements, body fat prediction equations have been developed that are age and gender specific.

Circumference measurements of the waist (abdominal circumference) and hips (gluteal circumference) also provide an index of regional body fat distribution and have been shown to correlate with visceral fat [24]. The ratio of waist to hip circumference is calculated following measurement of the subject's waist and hip. Waist measurements should be made below the rib cage and above the umbilicus in a horizontal plane at the narrow site, or the site of least circumference. Hip circumference should be measured at the site with the greatest circumference around the hips or buttocks. Soft tissue should not be compressed or indented during these measurements, and all measurements should be taken with the subject standing. Reproducibility of circumference measurements is good at 2% [24]. Ratios >0.8 in women and >0.95 in men are thought to indicate increased health risk. Waist circumferences >40 in (men) and >35 in (women), without comparison to hip circumference, also may be used to identify increased abdominal fat and thus increased risk for the development of obesity-associated conditions.

DENSITOMETRY/HYDRODENSITOMETRY

The principle of hydrostatic weighing on which densitometry or hydrodensitometry is based can be traced to the

Greek mathematician Archimedes. He discovered that the volume of an object submerged in water is equal to the volume of water displaced by the object. The specific gravity or density of an object can be calculated by dividing the object's weight (wt) in air by its loss of weight in water. For example, for a person who weighs 47 kg in air and 2 kg underwater, 45 kg represents the loss of body weight and the weight of the water displaced. Correction for residual air volume in the lungs (RLV) and gas in the gastrointestinal tract (GIGV) must be made.

The calculation of body density is given by the following formula:

$$\text{Body Density} = \frac{\text{Wt of body in air}}{\frac{(\text{Wt of body in air} - \text{Wt of body underwater})}{\text{Density of Water}} - \text{RLV} - \text{GIGV}}$$

Residual lung volume is thought to be about 24% of vital lung capacity. The volume of gas in the gastrointestinal tract is estimated to range from 50 to 300 mL. This volume typically is neglected, or a value of 100 mL may be used in calculations. The density or the weight of water is known for a wide range of temperatures and must be obtained for the calculation.

Calculating the density of the human body allows an estimation of body fat. At any known body density, estimating the percentage of body fat in an adult is possible using an equation derived by Siri [25]:

$$\text{Percentage of body fat} = \frac{495}{\text{Body Density}} - 450 \times 100$$

or an equation derived by Brozek [26]:

$$\text{Percentage of body fat} = \frac{457}{\text{Body Density}} - 414 \times 100$$

Calculations of body density are derived in part from the knowledge that the density of fat mass is 0.9 g/cm³ and that the density of fat-free mass is 1.1 g/cm³ (assuming fat-free mass is composed of about 20.5% protein, 72.4% water, and 7.1% bone mineral). Once the percentage of body fat has been calculated, the weight of the fat and the lean body mass can be estimated as follows [20]:

$$\text{Body weight} \times \text{percentage body fat} = \text{Weight of body fat}$$

$$\text{Body weight} - \text{weight of body fat} = \text{Lean body weight}$$

Various calculations for determining ideal or desirable body weight have been proposed based on information of body composition, such as this formula [27]:

$$\text{Desirable body weight} = \frac{\text{Lean body weight}}{1 - \text{percent fat desired}}$$

Calculations would be as follows for a woman who weighs 200 lb, with a measured 40% of this weight as fat:

$$200 \text{ lb} \times 0.40 = 80 \text{ lb} \text{ (fat weight)}$$

$$200 \text{ lb} - 80 \text{ lb} = 120 \text{ lb} \text{ (lean body weight)}$$

Because the desirable amount of fat in females ranges from about 20% to 30%, a figure of 25% (0.25) is used in the following equation for the sample woman:

$$\text{Desirable body weight} = \frac{120}{1 - 0.25} = \frac{120}{0.75} = 160 \text{ lb}$$

Underwater weighing is considered a noninvasive and relatively precise method for assessment of body fat. The standard error of estimate of body fat using densitometry has been estimated at 2.7% for adults and about 4.5% for children and adolescents [27]. Limitations of underwater weighing include its relatively high equipment cost, the inability to measure gas volume in the gastrointestinal tract, its impracticality for large numbers of subjects, and the extreme cooperation and time required of subjects, who must be submerged and remain motionless for an extended time. Thus, the technique is not suitable for young children, older adults, or subjects in poor health. Additional limitations to its use include its assumption that density of lean body mass is relatively constant, when in fact bone density typically changes with age [28].

AIR-DISPLACEMENT PLETHYSMOGRAPHY

Another way to determine the volume of the body is with air-displacement plethysmography (APD). In a commercially available apparatus (Bod Pod Life Measurements Inc.), the subject is seated in a sealed chamber of known volume. A second chamber is separated by a membrane. The instrument measures the change in pressure caused by the volume occupied by the person. The person is dressed in a tight-fitting bathing suit and wears a bathing cap (to displace pockets of air in the hair). The measurement takes only a few minutes to complete. Measurements obtained by underwater weighing correlate well in broad populations with those obtained by other techniques. Some studies have reported some variations or underestimation of fat in specific groups when this method is compared with the dual-energy X-ray absorptiometry (DEXA) [29]. The apparatus has an advantage in that it can measure the body composition in age groups that are not suitable for underwater weighing, such as the elderly or the very young. An instrument called the PEA POD is designed for small children. Once the density of the body is obtained, the calculation of body composition is the same as with hydrodensitometry.

ABSORPTIOMETRY

Absorptiometry is an imaging technique that involves scanning the entire body or a portion of the body by a

photon beam. Single-photon absorptiometry involves scanning the body with photons from ^{125}I (iodine) at a specific energy level. However, this technique does not allow accurate measurement at soft tissue sites. This problem has been eliminated with the development of dual-photon absorptiometry. In dual-photon absorptiometry, the radio-nuclide source is generally ^{153}Gd (gadolinium), and photons at two different energy levels are emitted. Bone mineral content as well as fat mass and fat-free mass may be estimated from dual-photon absorptiometry.

Dual energy X-ray absorptiometry (abbreviated DEXA or DXA), introduced in the late 1980s, involves scanning subjects with X rays at two different energy levels. The subject lies on a table while a photon source beneath the table and the detector above the table pass across the subject's body. Attenuation of the beam of X rays as it passes over the body is calculated by computer. Percentage of body fat, soft tissue, and bone mineral density (total or specific sites) can be calculated based on the restriction in the flux of the X rays across the fat and the fat-free masses [23,30–32].

Limitations to the use of absorptiometry include the expense of the equipment and the exposure, though minimal, of subjects to radiation. In addition, trained personnel are required to run the instrument and analyze the scans. DXA measurements are highly reproducible and correlate with other body composition assessment methods. The technique, however, is not accurate for people with metal implants, including, for example, pins or rods. In addition, extremely obese people may have difficulty getting on the table, assuming the table can support the weight.

COMPUTERIZED (AXIAL) TOMOGRAPHY (CAT OR CT)

Computerized or computed (axial) tomography (CT or CAT), another imaging technique involving an X-ray tube and detectors aligned at opposite poles of a circular gantry, creates visual images and thus enables regional body composition (such as visceral organ mass; regional muscle mass; subcutaneous and internal fat; and bone density) to be determined. Subjects lie face-up on a movable platform that passes through the instrument's circular gantry. Cross-sectional images of tissue are constructed by the scanner computer as the X-ray beam rotates around the person being assessed. Differences in X-ray attenuation are related to differences in the physical density of tissues [23]. The relative surface area or volume occupied by tissues (e.g., bone, adipose, and fat-free tissue) can be calculated from the images produced by the instrument. Results are highly reproducible [24]. Excessively long exposure of subjects to ionizing radiation and the expense of the equipment are major drawbacks to using computerized tomography to assess body composition.

MAGNETIC RESONANCE IMAGING (MRI)

Magnetic resonance imaging (MRI) is based on the principle that atomic nuclei behave like magnets when an external magnetic field is applied across the body. When an external magnetic field is applied, the nuclei attempt to align with the field. The nuclei also absorb radio frequency waves directed into the body and in turn change their orientation in the magnetic field [23]. Abolishing the radio wave results in the emission of a radio signal by the activated nuclei, and this emitted signal is used to develop a computerized image. Magnetic resonance imaging is used to measure organ size and structure, body fat and fat distribution (subcutaneous, visceral, intra-abdominal), and muscle size, as well as body water contents. The technique is noninvasive and safe; however, the cost is quite high. Reproducibility of visceral fat area measured by magnetic resonance imaging is about 10% to 15% [24]. However, for assessment of adipose tissue distribution, magnetic resonance imaging provided the least variability when compared to skin fold thickness, ^{40}K counting (see the section “Total Body Potassium”), bioelectrical impedance, total body water assessment with ^{18}O (see the section “Total Body Water”), and hydrostatic weighing [33].

TOTAL BODY ELECTRICAL CONDUCTIVITY (TOBEC)

The total body electrical conductivity (TOBEC) technique is based on the change in electrical conductivity when a subject is placed in an electromagnetic field. Subjects lie face-up on a bed, which is rolled into the TOBEC instrument. The instrument then induces an electrical current in the subject. Changes in conductivity, which are proportional to the body's electrolyte content, are measured. Because electrolytes in the body are associated mostly with lean body mass, TOBEC enables lean body mass to be estimated and thus fat to be determined by difference. Hydration status, electrolyte imbalances, and variations in bone mass may, however, interfere with accuracy. In addition, although the procedure is fast and safe, the equipment is very expensive.

BIOELECTRICAL IMPEDANCE ANALYSIS (BIA)

Bioelectrical impedance analysis (BIA), also called bioelectrical impedance (BEI), is similar to TOBEC in that it depends on changes in electrical conductivity. However, in BIA, measurement of electrical conductivity is made on the extremities and not on the whole body. Subjects lie face-up on a bed, with their extremities away from the body. Electrodes are placed on the limbs in specific locations. An instrument generates a current or multiple electrical current frequencies that are passed through the body by means of the electrodes. Opposition to the electric flow

current, called impedance, is detected and measured by the instrument. Impedance is the inverse of conductance. The lowest resistance value of a person is used to calculate conductance and predict lean body mass or fat-free mass. For example, muscle, organs, and blood, which have high water and electrolyte content, are good conductors [34]. Tissues containing little water and electrolytes (such as adipose tissue) are poor conductors and have a high resistance to the passage of electrical current [34]. At higher frequencies, estimates of both intracellular and extracellular water can be made, because the current can penetrate cell membranes. At lower frequencies, the flow of the current is blocked, and the measured resistance indicates extracellular water.

Bioelectrical impedance is a safe, noninvasive, and rapid means to assess body composition. The equipment is portable and fairly easy to operate, although it is also relatively expensive. Like TOBEC, bioelectrical impedance readings are affected by hydration and electrolyte imbalances. Thus, the technique is more useful for healthy subjects. Several bioelectrical impedance analysis prediction equations have been developed for various populations.

ULTRASONOGRAPHY OR ULTRASOUND

Ultrasound provides images of tissue configuration or depth readings of changes in tissue density [23]. Electrical energy is converted in a probe to high-frequency ultrasonic energy. The ultrasonic energy is transmitted through the skin and into the body in the form of short pulses or waves. The waves pass through adipose tissue until they reach lean body mass. At the interface between the adipose and lean tissues, part of the ultrasonic energy is reflected back to the receiver in the probe and transformed to electrical energy. The echo is visualized on an oscilloscope. A transmission gel used between the probe and the skin provides acoustic contact. The equipment is portable, and the technique may provide information on the thickness of subcutaneous fat as well as the thickness of muscle mass. Reliability of the technique has improved to 91%, and accuracy is similar to that of skin fold measurements [35].

INFRARED INTERACTANCE

Infrared interactance is based on the principle that when material is exposed to infrared light, the light is absorbed, reflected, or transmitted depending on the scattering and absorption properties of the material. To assess body composition, a probe that acts as an infrared transmitter and detector is placed on the skin. Infrared light of two wavelengths is transmitted by the probe. The signal penetrates the underlying tissue to a depth of 4 cm [23]. Infrared light also is reflected and scattered at the site from the skin and underlying subcutaneous tissues and detected by the probe. Estimates of body composition can be made by analyzing

specific characteristics of the reflected light. The method is safe, noninvasive, and rapid; however, overestimates of body fat in lean (<8% body fat) subjects and underestimates of body fat in obese (>30% body fat) subjects have been reported [36]. The accuracy of the technique requires further investigation.

Some methods of body composition assessment use an atomic perspective to quantify one or more components of the body and through various calculations determine the other body components. Measurements of body composition by assessment of total body water, total body potassium, or neutron activation analysis use such an approach.

TOTAL BODY WATER (TBW)

Quantification of total body water involves the use of isotopes—typically deuterium (D_2O), radioactive tritium (3H_2O), or oxygen-18 (^{18}O)—and is based on principles of dilution. Water can be labeled with any one of the three isotopes. The water containing a specific amount (concentration) of the isotope is then ingested or injected intravenously. Following ingestion or injection, the isotope distributes itself throughout the body water. Body water occupies about 73.2% of fat-free body mass. After allowing a specified time period (usually 2 to 6 hours) for equilibration, samples of body fluids (usually blood and urine) are taken. Losses of the isotope in the urine must be determined. If ^{18}O is used, breath samples are collected for analysis. Concentrations of the isotope in the breath or body fluids are determined by scintillation counters or other instruments.

The initial concentration (C_1) and volume (V_1) of the isotope given are equal to the final concentration of the isotope in the plasma (C_2) and the volume of total body water (V_2), expressed as $C_1V_1 = C_2V_2$. Thus, total body water (V_2) is equal to C_1V_1 divided by C_2 . Once total body water has been determined, the percentage of lean body mass can be calculated. Fat-free mass equals total body water divided by 0.732. Body fat can be obtained by subtracting fat-free mass from body weight. A three-component model (consisting of total body water, body volume, and body weight) has been shown to measure changes in body fat as low as 1.54 kg in people [30]. Many studies, however, have shown that the degree of hydration varies considerably in lean body tissue of apparently healthy people. Therefore, implications about total body fat derived from estimates of lean body mass based on total body water may be misleading [26,37]. In addition, adipose tissue has been shown to contain as much as 15% water by weight [31]. Thus, extracellular fluid, as well as total body water, should be measured and subtracted from total body water to give an indication of intracellular water and thus of body cell mass.

Because total body water involves radiation exposure if 3H is used, the method is not suitable for use with some subjects (such as children and pregnant women). ^{18}O is in

itself quite expensive and requires expensive mass spectrometry equipment for its analysis. Deuterated water (D_2O) is relatively inexpensive to purchase but is expensive to measure [34].

TOTAL BODY POTASSIUM (TBK)

Total body potassium is also used to assess fat-free mass. Potassium is present within cells but is not associated with stored fat. About 0.012% of potassium occurs as ^{40}K , a naturally occurring isotope that emits a characteristic gamma ray. External counting of gamma rays emitted by ^{40}K provides the amount of total body potassium; however, getting accurate counts of ^{40}K may be difficult because of external or background radiation. After measurement of potassium (^{40}K) radiation from the body, calculation of total body potassium from the data is required. Fat-free mass can be estimated from the total body potassium based on any one of several conversion factors, which vary in men from 2.46 to 3.41 g potassium per kilogram fat-free mass and in women from 2.28 to 3.16 g potassium per kilogram fat-free mass [31]. Total body fat can be calculated by subtracting fat-free mass from body weight. Overestimation of body fat in obese subjects has been reported with total body potassium [36]. The technique should not be used in people with potassium-wasting diseases.

NEUTRON ACTIVATION ANALYSIS

Neutron activation analysis enables *in vivo* estimation of body composition, including total body concentrations of

nitrogen (TBN), calcium (TBCa), chloride (TBCl), sodium (TBNa), and phosphorus (TBP), among other elements. A beam of neutrons is delivered to the person being assessed. The body's atoms (nitrogen, calcium, chloride, sodium, and phosphorus) interact with the beam of neutrons to generate unstable radioactive elements, which emit gamma-ray energy as they revert back to their stable forms. The specific energy levels correspond to specific elements, and the radiation's level of activity indicates the element's abundance [23].

Assessment of many body components may be conducted with neutron activation analysis. For example, measuring nitrogen makes lean body mass assessment possible, and subtracting lean body mass from total body water allows total body fat calculation [38,39]. Table 6.9 [39] reports body composition information derived from neutron activation for males and females age 20 to 79 years. Neutron activation analysis is noninvasive and provides reliable and reproducible data. However, the equipment is expensive and requires a skilled technologist, and subjects are exposed to considerable amounts of radiation.

OVERVIEW OF METHODS

Table 8.2 provides an overview of the methods described in this chapter as well as a few additional methods. In brief review, the methods available to assess lean body mass (LBM) or fat-free mass include neutron activation analysis, total body potassium, intracellular water (total body water minus extracellular water), total body electrical conductivity, and bioelectrical impedance analysis.

Table 8.2 Methods for Assessing Body Composition [31,35,38,39,44]

Method	Description of Method and Comments
Anthropometry	Skin fold thicknesses from a variety of locations, body weight, and limb circumferences can be used to calculate fat, fat-free mass, and muscle size. Measurements can be made in the field but require skilled technicians for accuracy. Skin folds can provide some information about regional subcutaneous fat as well as about total fat. Measurements may not be applicable to all population groups.
Densitometry	Measurements of total body density through determination of body volume by underwater weighing, helium displacement, or combination of water displacement by body and air displacement by head. Measurements can be used to determine body density, which in turn allows calculation of percentage of body fat and fat-free mass. Measurements are precise but must be conducted in laboratory; subject cooperation is necessary for underwater weighing. The method is not suitable for young children or the elderly.
Densitometry and air displacement plethysmography (ADP)	Measurement of total body density through determination of body volume by underwater weighing, helium or air displacement (ADP), or a combination of the two. Measurements can be used to determine body density, which in turn allows calculation of percentage of body fat and fat-free mass. Underwater weighing measurements are precise and considered the gold standard for determining body composition. It must be conducted in a laboratory with subject cooperation. Underwater weighing is not suitable for young children and the elderly. Values of body density obtained by ADP correlate well with underwater weighing for most populations. With the commercial availability of equipment it can be used for infants, young children and the elderly.
Total body water (TBW)	Measured by dilution with deuterium (D_2O), tritium (3H_2O), or oxygen-18 (^{18}O). TBW is used as index of human body composition based on findings that water is not present in stored triglycerides but occupies an approximate average of 73.2% of the fat-free mass. A specified quantity of the isotope is ingested or injected; then, following an equilibration period, a sampling is made of the concentration of the tracer in a selected biological fluid. TBW is calculated from the equation $C_1V_1 = C_2V_2$ where V_2 represents TBW volume, C_1 is the amount of tracer given, and C_2 is the final concentration of tracer in the selected biological fluid. The ECF can be estimated by a variety of methods. Subtracting BCF from TBW allows calculation of fat-free mass. This is a difficult procedure with limited precision, and the cost can be great, particularly when ^{18}O is used as the tracer.

(Continued)

Table 8.2 (Continued)

Method	Description of Method and Comments
Total body potassium	⁴⁰ K, a naturally occurring isotope, is found in a known amount (0.012%) in intracellular water and is not present in stored triglycerides. These facts allow fat-free mass to be estimated by the external counting of gamma rays emitted by ⁴⁰ K. Instrument for counting ⁴⁰ K is quite expensive and must be properly calibrated for precision. Method is limited to laboratories.
Urinary creatinine excretion	Creatinine is the product resulting from the nonenzymatic hydrolysis of free creatine, which is liberated during the dephosphorylation of creatine phosphate. The preponderance of creatine phosphate is located in the skeletal muscle; therefore, urinary creatinine excretion can be related to muscle mass. Drawbacks to this method include large individual variability of creatinine excretion due to the renal processing of creatinine and the effect of diet. The creatine pool does not seem to be under strict metabolic control and is to some degree independent of body composition. Another technical difficulty is control of accurately limited 24-hour urine collections.
3-methylhistidine excretion	3-methylhistidine has been suggested as a useful predictor of human body composition because this amino acid is located principally in the muscle and cannot be reused after its release from catabolized myofibrillar proteins (methylation of specific histidine residues occurs posttranslationally on protein). Some concern exists over the use of 3-methylhistidine as a marker of muscle protein because of the potential influence of nonskeletal muscle protein (skin and gastrointestinal [GI] tract proteins) turnover on its excretory rate. Additional problems with this method are the need for consumption of a relatively controlled meat-free diet and complete and accurate urine collections.
Electrical conductance (a) Total body electrical	Method is based on the change in electrical conductivity when subject is placed in an electromagnetic field. The change is proportional to the electrolyte content of the body, and because fat-free mass contains virtually all the water and conducting electrolytes of the body, conductivity is far greater in the fat-free mass than in the fat mass. From measurement, LBM can be calculated and fat estimated by difference. A primary drawback to this method is the expense of the instrument required; this measurement is a laboratory procedure limited primarily to large clinical facilities.
(b) Bioelectrical impedance analysis	This method is an adaptation of TOBEC; measurement of electrical conductivity is made on extremities rather than the whole body. Determinations of resistance and reactance are made, and the lowest resistance value for an individual is used to calculate conductance and to predict LBM. Equipment is portable and much less expensive than that required for TOBEC, yet precision is comparable.
Absorptiometry (a) Single-photon	Method is used in measurement of local or regional bone. The bone is scanned by a low-energy photon beam and the transmission monitored by a scintillation detector. Changes in transmission as the beam is moved across the bone are a function of bone mineral content (bone density) in that region. Disadvantages of this method are that the bone must be enclosed in a constant thickness of soft tissue and that measurements cannot be used to accurately predict total skeletal mass.
(b) Dual-photon	This method allows estimation of LBM as well as total bone mineral of the whole body. The body is scanned transversely in very small steps over its entire length by radiation from gadolinium-153 (¹⁵³ Gd). This isotope emits two gamma rays of different energies; attenuation measurements at the two discrete photon energies allow quantification of bone mineral and soft tissue. The equipment required for dual-photon absorptiometry is quite expensive, complicated calibration is required, and data collected require complicated mathematical treatment.
(c) Dual X-ray photon	Similar to dual-photon, this method involves scanning subjects at two different energy levels; however, X rays are used instead of a radionuclide source. Radiation exposure to subjects is very low, and the procedure is relatively quick. This method appears to be the best choice for measuring bone mineral density.
Computerized tomography	Method determines regional body composition. An image is generated by computerized processing of X-ray data. Fat, lean tissue, and bone can be identified by their characteristic density-frequency distribution. Information about regional fat distribution can be obtained; it has been used to determine the ratio of intra-abdominal to subcutaneous fat in humans. The size of the liver, spleen, and kidney can be determined by computerized tomography. Both cost of the equipment and technical difficulties are high. Method is a laboratory procedure presently limited primarily to large medical centers.
Ultrasound	Approach uses instrument in which electrical energy is converted in probe to high-frequency ultrasonic energy. Subsequent transmission of these sound waves through various tissues can be used to calculate tissue thickness. Method is frequently used to determine the thickness of subcutaneous fat layer. Large laboratory instruments and smaller portable equipment are available. Although data suggest a reasonable validity of method, its general use has been limited because the appropriate signal frequency of the probe has not been well defined and the needed constant pressure by the probe to the scan site is difficult to achieve. Changes in pressure by probe application can prejudice ultrasonic determination of adipose tissue thickness.
Infrared interactance	Measurement of body fat is made at various sites on the extremities through use of short wavelengths of infrared light. The amount of fat can be calculated from the absorption spectra and used with a prediction equation to estimate TBF.
Magnetic resonance imaging	Approach is based on fact that atomic nuclei can behave like magnets. When external magnetic field is applied across a part of the body, each nucleus attempts to align with the external magnetic field. If these nuclei are simultaneously activated by a radio frequency wave, once the radio wave is turned off the activated nuclei will emit the signal absorbed; this emitted signal is used to develop image by computer. Method has capability of generating images in response to intrinsic tissue variables and of representing characteristics such as level of hydration and fat content. This method appears to have much potential, but both the cost of equipment and technical difficulties are high.
Neutron activation analysis	This is the only technique currently available for measurement of multielemental composition of the human body. Low radiation doses produce isotopic atoms in tissues; the induced nuclides permit measurement of many elements, including nitrogen, calcium, phosphorus, magnesium, sodium, and chloride. Although precision of measurement is high, so are the technical difficulties and cost of equipment. Method of measuring body composition is limited to a very few laboratories in this country and abroad.

Body fat may be assessed by anthropometry, densitometry, air-displacement plethysmography, dual-photon or energy X-ray absorptiometry, ultrasound, and infrared interactance. Adipose tissue typically is measured using computerized tomography, magnetic resonance imaging, and ultrasound. Bone mineral density may be assessed using single- or dual-photon absorptiometry or by dual energy X-ray absorptiometry [21,25,28,34].

Obesity is associated with an increased risk of morbidity and mortality. Conditions associated with being overweight or obese include hypertension, stroke, coronary artery disease, dyslipidemia, type 2 diabetes, sleep apnea, osteoarthritis, and numerous others. Energy balance is an area of utmost importance for those interested in the subject of nutrition and metabolism.

Energy Balance

The body is in energy balance when the energy intake is equal to the energy output. Energy input (or intake) is simpler to define than energy output. Energy intake is the sum of the energy in all of the food and beverages consumed. The energy is derived from the oxidation or breakdown of carbohydrates, protein, fats, and alcohol in our bodies. Energy output is more complex. Energy output includes the energy involved in the absorption, metabolism, and storage of the nutrients in the food we eat as well as the energy we spend as we breathe, our hearts beat, our bodies cool or warm, and we perform physical exercise. The regulation of food intake, expenditure of energy, and the storage of energy are very complex, and all aspects are not fully understood.

Consistent imbalance of energy results in either a gain or loss of body weight. If too little energy (calories) is consumed to balance energy expended, the amount of tissue in our body is reduced. The desired goal of a weight reduction is to lose adipose tissue, but other tissue such as muscle can be lost. If the energy consumed is larger than our expenditure, the adipose stores are increased; and if the positive balance is large enough for a long enough time we can become overweight or obese. Remember that obesity is defined as an excess of body fat and that we use body weight as a convenient proxy. For the nonathlete, body fat and body weight are well correlated.

For the past 20 years or so, prevalence of overweight and obesity has increased rapidly. This increase is considered to be an obesity epidemic by public health officials.

PREVALENCE OF OBESITY

An innate characteristic of maturation and aging is a change in body composition and body weight. These compositional changes occur throughout the life cycle, beginning with the embryo and extending through old age. Rapid growth entails not only an increase in body mass but also a change in the proportions of components making up this mass. Young adulthood is a period of relative homeostasis, but in some people, body composition can change. Following the more or less homeostatic period of young adulthood is the period of progressive aging, when some undesirable changes in body composition and often weight inevitably occur.

Obesity has been present throughout history, but recently it has reached epidemic proportions [40]. The data on the prevalence of obesity in United States has been mostly obtained from the National Health Examination Surveys (NHANES). NHANES has been a series of surveys (now continuous) that samples the United States population to permit prevalence calculations of a variety of nutrition and health parameters. The prevalence of obesity was stable from 1960 to 1980 [41]. During the NHANES I and II surveys, conducted during 1971–1974 and 1976–1980, respectively, obesity ($BMI \geq 30$) was just over 12% in men and 16% in women between 20 and 74 years. In the NHANES III survey, conducted between 1988–1994, the rate of obesity jumped to 20.6% in men and 25.9% in women. Starting with the continuous surveys all ages were sampled. Figure 8.5 shows the prevalence of overweight, obesity, and extreme obesity in adults over the age of 20 years. The definitions of overweight and obesity have been provided earlier in this chapter. The overall percentage of overweight and obese people stayed relatively

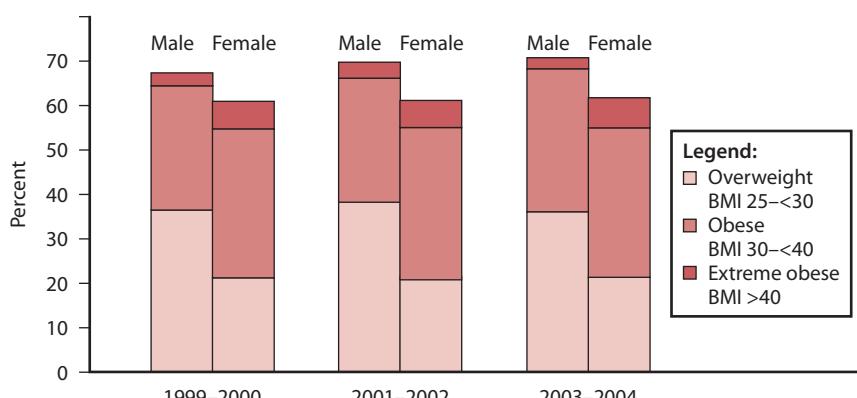


Figure 8.5 Prevalence of overweight and obesity in the United States, 1999–2004.

stable during the three surveys: about 70% of men and 61% of women. However, in these surveys more men were overweight and more women were obese. More women (6.5%–6.9%) than men (2.8%–3.6%) also were classified as extremely obese [42].

Similar increases have been observed in people younger than 20 years [41–43]. The prevalence of the risk of being overweight and obesity (definitions provided earlier in this chapter) among children had increased to greater than 33% by the 2003–2004 survey. Full coverage of the emergence of childhood obesity goes beyond the scope of this text. However, good evidence exists that an obese child is likely to become an obese adult. The morbidity and mortality related to the prevalence of overweight and obesity in the next generation of adults is likely to become even worse than it is now.

Total energy expenditure, which influences body weight and composition, is made up of the resting energy expenditure, the thermal effect of foods, and energy expenditure of physical activity (EEPA). Nutrition professionals need to know recommended body weight and body composition, and to assess energy expenditure to determine nutrient needs and identify or prevent disease.

Components of Energy Expenditure

Whether body weight is being maintained, increased, or decreased depends primarily on the extent to which the energy requirements of the body (i.e., total energy expenditure) have been met or exceeded by energy intake. Total energy expenditure is composed primarily of:

- the resting energy expenditure (REE), or basal metabolic rate (BMR)
- the thermic effect of food (TEF)
- the energy expenditure physical activity or exercise

A fourth component, thermoregulation, is sometimes included. The average division of energy expenditure among the components, each of which is described in the following sections, is shown in Figure 8.6.

BASAL METABOLIC RATE AND RESTING ENERGY EXPENDITURE

Basal metabolic rate (BMR) represents the rate at which the body expends energy to sustain basic life processes such as respiration, heartbeat, renal function, and blood circulation. It also includes the energy needed to remain in an awake state, because the measurements are usually made shortly after the person wakes. The word *basal*, as it is used in BMR, is often confused with the term *resting*; however, *basal* is more precisely defined than is *resting*.

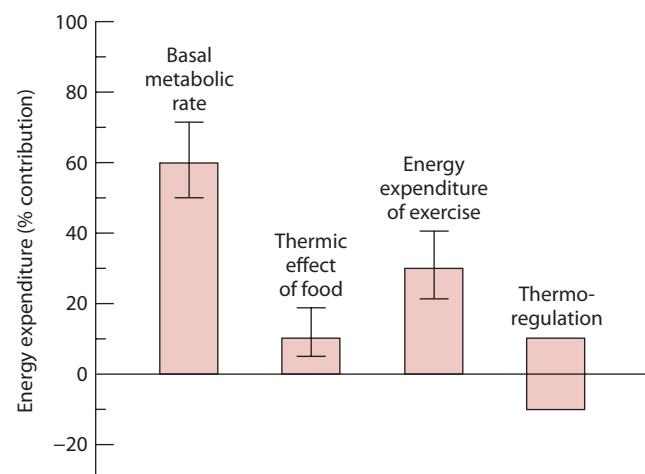


Figure 8.6 Components of energy expenditure and their approximate percentage contribution.

The measurement of oxygen consumed and carbon dioxide produced that is used in calculating energy expenditure is made under closely controlled and standardized conditions. A person's basal metabolic rate is determined when he or she is in a postabsorptive state (i.e., no food intake for at least 12 hours), is lying down (supine), and is completely relaxed (motionless)—preferably very shortly after awakening from sleep in the morning. In addition, the temperature of the room in which the measurement takes place is made as comfortable as possible (thermoneutral) for the person. Any factors that could influence the person's internal work are minimized as much as possible. For most people, energy expenditure is slowest during sleep. BMR is usually converted to units of kcal/24 hours and called *basal energy expenditure* (BEE).

In contrast to BMR, resting metabolic rate (RMR) is measured when the person is at rest in a comfortable environment. Fasting for 12 hours is not required. Instead, the fast for RMR is usually about 2 to 4 hours. RMR usually is slightly higher (about 10%) than BMR because of its less stringent conditions of measurement [44,45]. RMR is thought to account for about 65% to 80% of daily total energy expenditure [44]. BMR accounts for about 50% to 70% of daily total energy expenditure [46]. The term *resting energy expenditure* (REE) is used when RMR is extrapolated to units of kcal/24 hours [47].

Basal metabolism is a result of energy exchanges occurring in all cells of the body. The rate of oxygen consumption, however, is most closely related to the actively metabolizing cells, that is, the body's lean body mass or fat-free mass [48,49]. In aging, for example, fat increases at the expense of fat-free mass, and BMR decreases. With maturation, the proportion of supporting structures (i.e., bone and muscle) increases more rapidly than does total body weight. Bone and muscle, though components of body cell mass, have a much lower metabolic activity at rest than organ tissues

but much greater activity than adipose tissue. This difference in the rate of weight accretion between the less active and the more active components of mass means a decrease in the overall metabolic activity of cell mass and a concurrent decrease in BMR per unit of body weight [48]. These changes that occur during maturation explain the lower REE of children as compared to very young infants.

A look at the metabolic activity among the different components of the cell mass in an adult male illustrates its variability. Under normal circumstances, about 5% to 6% of total body weight can be attributed to the weight of the brain, liver, heart, and kidney, whereas about 30% to 40% of body weight is attributable to muscle mass [48]. At the same time, the metabolic activity of these organ tissues accounts for about 60% of basal oxygen consumption, whereas muscle mass accounts for only about 25% [48]. Tissues such as bone, glands, intestine, and skin account for 33% of body weight and contribute 15% to 20% of metabolic activity. In contrast, fat usually accounts for at least 20% of body weight but contributes only 5% of metabolic activity. Thus, changes in BMR can occur whenever the proportions of body tissues change in relation to one another [48].

Thermic Effect of Food

A second component of energy expenditure is the thermic effect of food (i.e., the metabolic response to food), also called diet-induced thermogenesis, specific dynamic action, or the specific effect of food. The thermic effect of food represents the increase in energy expenditure associated with the body's processing of food, including the work associated with the digestion, absorption, transport, metabolism, and storage of energy from ingested food. The percentage increase in energy expenditure over BMR caused by the thermic effect of food has been estimated to range from about 5% to 30%.

Protein in foods has the greatest thermic effect, increasing energy expenditure 20% to 30%. Carbohydrates have an intermediate effect, raising energy expenditure 5% to 10%, and fat increases energy expenditure 0% to 5% [47]. The value most commonly used for the thermic effect of food is 10% of the caloric value of a mixed diet consumed within 24 hours [46,45]. The rise in metabolism following food consumption appears to reach a maximum about 1 hour after eating and is generally, but not always, absent 4 hours postprandial (after eating) [45]. Consequently, the thermic effect of food often is not included in calculations of total energy requirements.

Energy Expenditure of Physical Activity

The energy expenditure of physical activity (i.e., voluntary movement, including fidgeting) is the most variable of the components and also the only component that is easily altered. Although on average, physical activity

accounts for about 20% to 40% of total energy expenditure, it can be considerably less in a truly sedentary person or much more in a very physically active person [46,50]. Factors impinging on energy expenditure during exercise, other than the activity itself, include the intensity, duration, and frequency with which the activity is performed; the body mass of the person; his or her efficiency at performing the activity; and any extraneous movements that may accompany the activity. In addition, oxygen consumption and thus energy expenditure can remain elevated for a short time period after the exercise activity has stopped.

THERMOREGULATION

An additional component of energy expenditure that is of some importance is thermoregulation, also called adaptive, nonshivering, facultative, or regulatory thermogenesis. Thermoregulation refers to the alteration in metabolism that occurs in the body's maintaining its core temperature. Changes in metabolism occur most often with changes in environmental temperature, especially below the comfort zone (zone of thermoneutrality), but changes in metabolism can also occur with overfeeding, trauma, burns, and physical conditioning, among other situations [47]. For example, when temperatures decrease below the comfort zone and a person has not adjusted to the change by altering the thickness of the clothes he or she is wearing, energy expenditure increases to maintain or restore the body temperature to normal. The overall contribution of thermoregulation to energy needs is small, given that most people alter clothing as needed to maintain a comfortable body temperature.

Assessing Energy Expenditure

In healthy adults, total energy expenditure typically includes basal energy expenditure, energy expended because of the thermic effect of foods, and thermic effects of physical activity. Energy expenditure can be assessed through direct or indirect calorimetry or the doubly labeled water method as well as by several calculations using derived formulas. Each of these methods of assessment is explained below.

DIRECT CALORIMETRY

Measurement of total energy expenditure can be determined by **direct calorimetry**, which measures the dissipation of heat from the body [51]. Heat dissipation is measured using an isothermal principle, a gradient-layer system, or a water-cooled garment [51,52]. A very simplified version of calorimetry for humans based on the isothermal

principle is depicted in Figure 8.7. Total heat loss consists of sensible heat loss and heat of water vaporization. In the isothermal calorimeter (Figure 8.7), sensible heat loss is determined by the difference in water temperature and in the amount of water flowing in and out of pipes situated within the walls of the chamber where the subject has been placed. Heat removed by the vaporization of water is calculated from the moisture of the air leaving the calorimeter. The total moisture of the air is absorbed in a sulfuric acid bath. Although the concept of direct calorimetry is relatively simple, direct measurement of body heat loss is expensive, cumbersome, and usually rather unpleasant for the subject or subjects involved [50]. Thus, basal metabolic rate is usually measured indirectly.

INDIRECT CALORIMETRY

Indirect calorimetry measures the consumption of oxygen and the expiration of carbon dioxide. Urinary nitrogen excretion should also be measured, because for every 1 g of nitrogen excreted, about 6 L of oxygen is consumed and 4.8 L of carbon dioxide is produced [51,53]. Oxygen consumption and carbon dioxide production are measured either by using portable equipment that can be placed on a person, enabling collection and analysis of expired air and quantification of inspired air, or with stationary equipment. The exchange of oxygen and carbon dioxide is proportional to metabolism.

The amount of energy expended can be calculated from the ratio of the carbon dioxide expired to the oxygen inhaled. This ratio is known as the respiratory quotient (RQ). Exercise physiologists use the term respiratory exchange ratio (R). Examination of respiratory

quotients provides meaningful information with respect to both energy expenditure and the biological substrate (carbohydrate or fat) being oxidized. However, with regard to substrate oxidation, no information about metabolism in individual organs and tissues is gained [51]. The next sections present a brief explanation of how the respiratory quotient is used to assess substrate oxidation and how it is used to determine energy expenditure.

The Respiratory Quotient and Substrate Oxidation

An RQ equal to 1.0 suggests that carbohydrate is being oxidized, because the amount of oxygen required for the combustion of glucose equals the amount of carbon dioxide produced, as shown here:



$$\text{RQ} = 6 \text{ CO}_2 / 6 \text{ O}_2 = 1.0$$

The RQ for a fat is <1.0 because fat is a much less oxidized fuel source (fewer oxygen molecules). For example, a triacylglycerol such as tristearin, as shown in the following reaction, requires 163 mol oxygen and produces 114 mol of carbon dioxide:



$$\text{RQ} = 114 \text{ CO}_2 / 163 \text{ O}_2 = 0.70$$

Calculating the RQ for protein oxidation is more complicated, because metabolic oxidation of amino acids requires removing the nitrogen and some oxygen and carbon as urea, a compound excreted in the urine. Urea nitrogen represents a net loss of energy to the body, and only the remaining carbon chain of the amino acid can be oxidized in the body. The following reaction illustrates the oxidation of a small protein molecule into carbon dioxide, water, sulfur trioxide, and urea:



$$\text{RQ} = 63 \text{ CO}_2 / 77 \text{ O}_2 = 0.818$$

The average figures 1.0, 0.7, and 0.8 are accepted as the representative RQs for carbohydrate, fat, and protein, respectively. The RQ for an ordinary mixed diet consisting of the three energy-producing nutrients is usually considered to be about 0.85. An RQ of 0.82 represents the metabolism of a mixture of 40% carbohydrate and 60% fat [26]. RQs that are actually computed from gaseous exchange and that come closer to 1.0 or nearer to 0.7 would indicate that more carbohydrate or fat, respectively, was being used for fuel. In clinical practice, an RQ <0.8 suggests that a patient may be underfed, and an RQ <0.7 suggests starvation or ingestion of a low-carbohydrate or high-alcohol diet; whereas an RQ 1.0 suggests that lipogenesis is occurring [51].

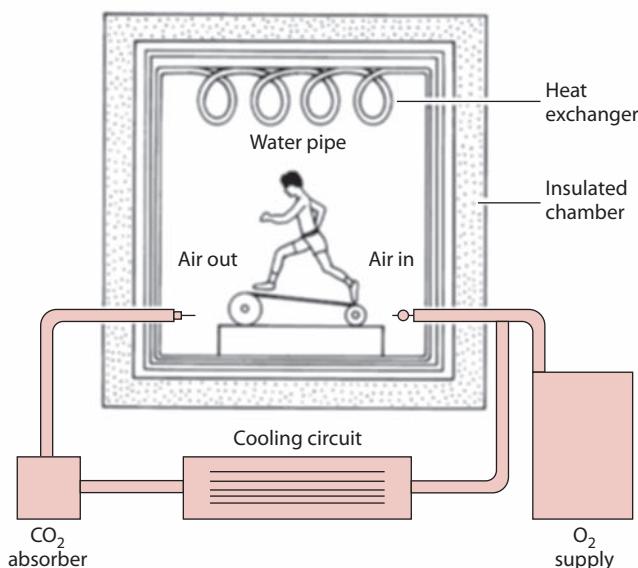


Figure 8.7 A simplified version of the human calorimeter used to measure direct body heat loss (i.e., energy expenditure).

The Respiratory Quotient and Energy Expenditure

Once the RQ has been computed from gaseous exchange, the calculation of energy expended is rather simple. Table 8.3 gives the caloric value for 1 L of oxygen and for 1 L of carbon dioxide, given various RQs. When the volume of oxygen or carbon dioxide in the exchange has been determined, the total caloric value represented by the exchange can be calculated. Determining the amount of carbohydrate and fat being oxidized in the production of these calories is also possible.

For example, if under standard conditions for the determination of BMR a person consumed 15.7 L oxygen per hour and expired 12.0 L carbon dioxide, the RQ would be $12.0/15.7$, or 0.7643. From Table 8.3, the caloric equivalent for an RQ of 0.76 is 4.751 kcal for 1 L oxygen or 6.253 kcal

for 1 L carbon dioxide. Based on the caloric equivalent for oxygen, calories produced per hour are 15.7×4.751 or 74.59 kcal. Based on the caloric equivalent for carbon dioxide, calories produced per hour are 12.0×6.253 , or 75.04 kcal. If we use 75 kcal/h as the caloric expenditure under basal conditions, the basal energy for the day would be about 1,800 kcal ($75 \text{ kcal/h} \times 24 \text{ h}$). At this RQ of 0.76, fat is supplying almost 81% of energy expended (Table 8.3).

Because under ordinary circumstances the contribution of protein to energy metabolism is so small, the oxidation of protein is ignored in the determination of the so-called nonprotein RQ. If a truly accurate RQ is required, a minimal correction can be made by measuring the amount of urinary nitrogen excreted over a specified time period. As you have read, for every 1 g of nitrogen excreted, about 6 L oxygen is consumed and 4.8 L carbon dioxide is produced. The amount of oxygen and carbon dioxide exchanged in the release of energy from protein can then be subtracted from the total amount of measured gaseous exchange.

Measurement of the energy expended in various activities has also been made primarily through indirect calorimetry. The method for measuring gas exchange, however, differs from that used for determining BMR. The subject performing the activity for which energy expenditure is being determined inhales ambient air, which has a constant composition of 20.93% oxygen, 0.03% carbon dioxide, and 78.04% nitrogen. Air exhaled by the subject is collected in a spirometer (a device used to measure respiratory gases) and is analyzed to determine how much less oxygen and how much more carbon dioxide it contains compared with ambient air. The difference in the composition of the inhaled air and the exhaled air reflects the energy release from the body [26]. A lightweight portable spirometer (Figure 8.8) can be worn during the performance of almost any sort of activity, and thus freedom of movement outside the laboratory is possible. In the laboratory, the Douglas bag is routinely used to collect expired air [26].

Tables are available that list the kilocalories expended per kilogram body weight per minute or hour for a wide variety of activities. Table 8.4, an example of such a table, groups various activities together according to their average level of energy expenditure. Information on the energy needs associated with physical activities, coupled with the information on basal or resting energy needs derived from indirect calorimetry, allows a person's total energy needs to be assessed. These tables must be used carefully to determine what time span is measured (per minute or per hour) and whether the number includes basal energy expenditure. Table 8.5 provides information on the energy expended on various activities and includes the energy used for basal metabolism for people of different body weights.

Table 8.3 Thermal Equivalent of O₂ and CO₂ for Nonprotein RQ

Nonprotein RQ	Caloric Value 1 L O ₂	Caloric Value 1 L CO ₂	Source of Calories	
			Carbohydrate (%)	Fat (%)
0.707	4.686	6.629	0	100
0.71	4.690	6.606	1.10	98.9
0.72	4.702	6.531	4.76	95.2
0.73	4.714	6.458	8.40	91.6
0.74	4.727	6.388	12.0	88.0
0.75	4.739	6.319	15.6	84.4
0.76	4.751	6.253	19.2	80.8
0.77	4.764	6.187	22.8	77.2
0.78	4.776	6.123	26.3	73.7
0.79	4.788	6.062	29.9	70.1
0.80	4.801	6.001	33.4	66.6
0.81	4.813	5.942	36.9	63.1
0.82	4.825	5.884	40.3	59.7
0.83	4.838	5.829	43.8	56.2
0.84	4.850	5.774	47.2	52.8
0.85	4.862	5.721	50.7	49.3
0.86	4.875	5.669	54.1	45.9
0.87	4.887	5.617	57.5	42.5
0.88	4.899	5.568	60.8	39.2
0.89	4.911	5.519	64.2	35.8
0.90	4.924	5.471	67.5	32.5
0.91	4.936	5.424	70.8	29.2
0.92	4.948	5.378	74.1	25.9
0.93	4.961	5.333	77.4	22.6
0.94	4.973	5.290	80.7	19.3
0.95	4.985	5.247	84.0	16.0
0.96	4.998	5.205	87.2	12.8
0.97	5.010	5.165	90.4	9.58
0.98	5.022	5.124	93.6	6.37
0.99	5.035	5.085	96.8	3.18
1.00	5.047	5.047	100	0

Source: Adapted from McArdle, W.D., Katch, F.I., Katch, V.L., *Exercise Physiology*, 2nd ed., p. 127 (Philadelphia: Lea & Febiger, 1986).



Figure 8.8 Measurement of oxygen consumption by portable spirometer during (a) golf, (b) cycling, (c) sit-ups, and (d) calisthenics.

DOUBLY LABELED WATER

The doubly labeled water method also enables assessment of total energy expenditure. In this technique, stable isotopes of water are given as $H_2^{18}O$ and as 2H_2O (or as $^2H_2^{18}O_2$), and the disappearance of the $H_2^{18}O$ and 2H_2O is measured in the blood and urine for about 3 weeks. The $^{18}O_2$ can be excreted either as CO_2 or H_2O . The disappearance of the $H_2^{18}O$ is representative of the flux of water (i.e., water

turnover) and of the production rate of carbon dioxide. The 2H_2 can only be excreted as H_2O , and the disappearance of the 2H_2O represents water turnover alone. The difference between the disappearance rate of $H_2^{18}O$ and that of 2H_2O corresponds to the production rate of carbon dioxide. To assess oxygen consumption, a food quotient (FQ) is calculated from diet records kept throughout the testing period. In subjects maintaining body weight, the food quotient is equal to the respiratory quotient. Oxygen consumption,

Table 8.4 The Energy Cost above Basal Associated with Different Activities

Energy Level	Type of Activity	Energy (kcal/kg/minute*)	
		Woman	Man
a	Sleeping or lying still, relaxed†	0.000	0.000
b	Sitting or standing still (such as sewing, writing, eating)	0.001–0.007	0.003–0.012
c	Very light activity (driving a car, walking slowly on level ground)	0.009–0.016	0.014–0.022
d	Light exercise (sweeping, eating, walking normally, carrying books)	0.018–0.035	0.023–0.040
e	Moderate exercise (fast walking, dancing, bicycling, cleaning vigorously, moving furniture)	0.036–0.053	0.042–0.060
f	Heavy exercise (fast dancing, fast uphill walking, hitting tennis ball, swimming, gymnastics)	0.055	0.062

*Measured in kilocalories per kilogram per minute above basal energy. Where ranges are given, pick the midpoint within the range, unless you have reason to believe you are unusually relaxed or energetic when performing the activity. For example, for “sitting,” a man should normally pick 0.007; if he is sitting very relaxed, 0.003; if very tense, 0.012.

†For purposes of this table, these are assumed to be at the basal level of activity. Source: Modified from Whitney E, Cataldo C. *Understanding Normal and Clinical Nutrition*, St. Paul, MN: West, 1983.

and thus energy expenditure, can be calculated from the FQ and carbon dioxide production.

Use of the doubly labeled water method to assess total energy expenditure produces accurate results that correlate well with those of indirect calorimetry. The main source of error in the technique lies with the use of food records, which may not necessarily be accurate, and with the calculation of oxygen consumption from the food quotient [47].

DERIVED FORMULAS

In contrast to the doubly labeled water technique, which has been adapted for use in humans only since the 1980s, estimating basal metabolic rate or resting energy expenditure rather than measuring it has been the practice among clinicians since about 1925. Many different methods for estimating energy needs have been used over the years. Estimations have been based on body surface area, body weight, and calculations from regression equations that incorporate the person’s gender, age, weight, and height. Such estimates have been shown to correlate with measurements from indirect calorimetry or from doubly labeled water.

One estimate of basal metabolic rate for all mammals, including humans, is based on body weight raised to the

power of 0.75 [48,54]. The equation $BMR \text{ (kcal/day)} = 70 \times W^{0.75}$ uses weight (W) measured in kilograms and raised to the power of 0.75 multiplied by 70 for estimating BMR. Table 8.6 provides sample body weights raised to the power of 0.75 to aid the reader in performing calculations.

Because of the relatively narrow range of human body size, calculations from the preceding equation give an estimate that is reasonably close to the BMR value obtained from the formula of 1 kcal/kg/h for men and 0.9 kcal/kg/h for women.

Using the two formulas to estimate the BMR of a 70 kg man illustrates their comparable results:

$$1. BMR = 70 \times 70^{0.75} = 70 \times 24.2 = 1,694 \text{ kcal/day}$$

$$2. BMR = 1 \text{ kcal} \times 70 \text{ kg} \times 24 \text{ hours} = 1,680 \text{ kcal/day}$$

The derived regression equations probably most often used [55] to estimate BMR in the clinical setting are those derived by Harris and Benedict in 1919, based on indirect calorimetry [56] and only slightly modified. Using the Harris-Benedict equations, BMR (kilocalories per day) is predicted in separate equations for men and women based on weight in kilograms (W), height in centimeters (H), and age in years (A):

$$\begin{aligned} \text{Men: BMR} &= 66.5 + (13.7 \times W) + (5.0 \times H) \\ &\quad - (6.8 \times A) \end{aligned}$$

$$\begin{aligned} \text{Women: BMR} &= 655.1 + (9.56 \times W) + (1.85 \times H) \\ &\quad - (4.7 \times A) \end{aligned}$$

Other equations, by Mifflin and St. Jeor [57], predict REE (kilocalories per day) for men and women as follows:

$$\text{Men: REE} = (10 \times W) + (6.25 \times H) - (5 \times A) + 5$$

$$\text{Women: REE} = (10 \times W) + (6.25 \times H) - (5 \times A) - 161$$

A female who is 35 years old, weighs 125 lb (56.82 kg), and is 5 ft, 5 in tall (165.1 cm) would have a BMR of 1,339 kcal (using the Harris-Benedict equation) and an REE of 1,264 kcal (using the Mifflin-St. Jeor equation).

The various equations used to calculate energy expenditure are reevaluated regularly in scientific literature. Reevaluations have shown that predicted values for BMR are often higher than the actual expenditure and may not be applicable to all people, for example, those who are obese [55,58]. Thus, dietitians must be alert to the literature for recent findings and recognize the limitations and implications of the use of the various equations.

Using the Harris-Benedict or Mifflin-St. Jeor equations, among other formulas mentioned, provides information on a person’s basal or resting energy needs. To determine total energy needs for a particular person, the energy required for physical activity must be added to the energy needed for basal needs. Depending on the type, duration, intensity, and frequency of physical activity, energy needs for physical activity may vary from about 20% to 70% or more of basal metabolism. Activity factors can be multiplied by

Table 8.5 Energy Expended on Various Activities*

Activity	kcal/lb/min [†]	kcal/min at Different Body Weights				
		110 lb	125 lb	150 lb	175 lb	200 lb
Aerobic dance (vigorous)	0.062	6.8	7.8	9.3	10.9	12.4
Basketball (vigorous, full court)	0.097	10.7	12.1	14.6	17.0	19.4
Bicycling						
13 mph	0.045	5.0	5.6	6.8	7.9	9.0
15 mph	0.049	5.4	6.1	7.4	8.6	9.8
17 mph	0.057	6.3	7.1	8.6	10.0	11.4
19 mph	0.076	8.4	9.5	11.4	13.3	15.2
21 mph	0.090	9.9	11.3	13.5	15.8	18.0
23 mph	0.109	12.0	13.6	16.4	19.0	21.8
25 mph	0.139	15.3	17.4	20.9	24.3	27.8
Canoeing (flat water, moderate pace)	0.045	5.0	5.6	6.8	7.9	9.0
Cross-country skiing						
8 mph	0.104	11.4	13.0	15.6	18.2	20.8
Golf (carrying clubs)	0.045	5.0	5.6	6.8	7.9	9.0
Handball	0.078	8.6	9.8	11.7	13.7	15.6
Horseback riding (trot)	0.052	5.7	6.5	7.8	9.1	10.4
Rowing (vigorous)	0.097	10.7	12.1	14.6	17.0	19.4
Running						
5 mph	0.061	6.7	7.6	9.2	10.7	12.2
6 mph	0.074	8.1	9.2	11.1	13.0	14.8
7.5 mph	0.094	10.3	11.8	14.1	16.4	18.8
9 mph	0.103	11.3	12.9	15.5	18.0	20.6
10 mph	0.114	12.5	14.3	17.1	20.0	22.9
11 mph	0.131	14.4	16.4	19.7	22.9	26.2
Soccer (vigorous)	0.097	10.7	12.1	14.6	17.0	19.4
Studying	0.011	1.2	1.4	1.7	1.9	2.2
Swimming						
20 yd/min	0.032	3.5	4.0	4.8	5.6	6.4
45 yd/min	0.058	6.4	7.3	8.7	10.2	11.6
50 yd/min	0.070	7.7	8.8	10.5	12.3	14.0
Table tennis (skilled)	0.045	5.0	5.6	6.8	7.9	9.0
Tennis (beginner)	0.032	3.5	4.0	4.8	5.6	6.4
Walking (brisk pace)						
3.5 mph	0.035	3.9	4.4	5.2	6.1	7.0
4.5 mph	0.048	5.3	6.0	7.2	8.4	9.6
Wheelchair basketball	0.084	9.2	10.5	12.6	14.7	16.8
Wheeling self in wheelchair	0.030	3.3	3.8	4.5	5.3	6.0

*The values listed in this table reflect both the energy spent in physical activity and the amount used for BMR.

[†]To calculate kcalories spent per minute of activity for your own body weight, multiply kcal/lb/min by your exact weight and then multiply that number by the number of minutes spent in the activity. For example, if you weigh 142 lb, and you want to know how many kcalories you spent doing 30 min of vigorous aerobic dance: $0.062 \times 16 = 8.8 \text{ kcal/min}$, $8.8 \times 30 (\text{min.}) = 264 \text{ kcal spent total}$.

Source: From *Understanding Nutrition*, 9th edition by Whitney/Rolfes, 2002. Reprinted with permission of Brooks/Cole, a division of Thomson Learning: www.thomsonrights.com. Fax: 800-730-2215.

basal energy calculations to address the energy needs of physical activity.

Using the formulas, for example, a person with basal energy needs of 1,580 kcal who does little activity (i.e., sedentary) may expend only about 20% of kilocalories per day being physically active and thus would require 1,580 kcal basal + $(1,580 \text{ kcal} \times 0.20 \text{ activity}) = 1,580 \text{ kcal basal} + 316 \text{ kcal activity} = 1,896 \text{ kcal}$. This calculation also may be written as $1,580 \text{ kcal basal} \times 1.20 \text{ activity factor} = 1,896 \text{ kcal}$. Energy needed for the thermic effect of food is not included in many equations used to calculate total energy

needs, but when it is used, a value of 10% of basal energy needs is usually used. Thus, using the above example and including the thermic effect of foods (TEF) in the calculation, a person with basal energy needs of 1,580 kcal would need another $1,580 \text{ kcal basal} \times 0.10 \text{ TEF} = 158 \text{ kcal}$ for TEF. Total energy needs would be $1,580 \text{ kcal} + 316 \text{ kcal} + 158 \text{ kcal}$, or 2,054 kcal.

Based on data on total daily energy expenditure measured by the doubly labeled water method, the Food and Nutrition Board has developed several equations to calculate estimated energy requirements (EER). For an

Table 8.6 Body Weights in Kilograms Raised to the Power of 0.75

Weight (kg)	Weight ^{0.75} (kg)	Weight (kg)	Weight ^{0.75} (kg)
1	1.0	51	19.1
2	1.68	52	19.4
3	2.28	53	19.6
4	2.83	54	19.9
5	3.34	55	20.2
6	3.83	56	20.5
7	4.30	57	20.8
8	4.75	58	21.0
9	5.19	59	21.3
10	5.62	60	21.6
11	6.04	61	21.8
12	6.44	62	22.1
13	6.84	63	22.4
14	7.24	64	22.6
15	7.62	65	22.9
16	8.00	66	23.2
17	8.38	67	23.4
18	8.75	68	23.7
19	9.10	69	23.9
20	9.46	70	24.2
21	9.8	71	24.4
22	10.2	72	24.7
23	10.5	73	25.0
24	10.8	74	25.2
25	11.2	75	25.5
26	11.5	76	25.8
27	11.8	77	26.0
28	12.2	78	26.2
29	12.5	79	26.5
30	12.8	80	26.7
31	13.1	81	27.0
32	13.5	82	27.2
33	13.8	83	27.5
34	14.1	84	27.7
35	14.4	85	28.0
36	14.7	86	28.2
37	15.0	87	28.5
38	15.3	88	28.7
39	15.6	89	29.0
40	15.9	90	29.2
41	16.2	91	29.4
42	16.5	92	29.7
43	16.8	93	29.9
44	17.1	94	30.2
45	17.4	95	30.4
46	17.7	96	30.7
47	18.0	97	30.9
48	18.2	98	31.1
49	18.5	99	31.4
50	18.8	100	31.6

Source: Adapted from Pike, R., Brown, M., *Nutrition: An Integrated Approach*, 3rd ed., p. 749 (New York: Macmillan, 1984). Copyright © The McGraw-Hill Companies, Inc. Reproduced by permission of The McGraw-Hill Companies, Inc.

adult, “the estimated energy requirement is defined as the dietary energy intake that is predicted to maintain energy balance in a healthy adult of a defined age, gender, weight, height, and level of physical activity consistent with good health” [47].

Formulas to calculate the estimated energy requirements for adults follow:

$$\text{Adult men: EER} = 662 - (9.53 \times \text{age}) + \text{PA} (15.91 \times \text{weight} + 539.6 \times \text{height})$$

$$\text{Adult women: EER} = 354 - (6.91 \times \text{age}) + \text{PA} (9.36 \times \text{weight} + 726 \times \text{height})$$

where age is in years, weight is in kilograms (kg), and height is in meters (m) [54]. PA in the equation refers to physical activity coefficients and varies with the level of activity and with gender. PA values for different physical activity levels are given below.

PA Values for Different Physical Activity Levels [65]				
Gender	Sedentary	Low active	Active	Very Active
Men	1.00	1.11	1.25	1.48
Women	1.00	1.12	1.27	1.45

The appropriate PA value should be inserted into the EER equation to calculate total energy expenditure.

These formulas apply to adults with healthy body weights (BMI of 18.5 to 25 kg/m²). The formula for women applies to women who are not pregnant or lactating.

Regulation of Body Weight and Composition

Earlier, you read that the prevalence of obesity is increasing rapidly to what some public health experts are calling an epidemic. Recall that the goal of weight maintenance is to balance energy intake with energy expenditure. Weight maintenance is a lofty goal, but regulating food and beverage intake and energy expenditure is very complex, and this goal is not easy to achieve. Energy intake and energy expenditure clearly do not correlate over a short period of time such as a day or two. Equally clearly, however, their correlation over an extended period of time, such as a week to a few months to years, is excellent. If you consume as little as 10 kcal per day more than you expend, you gain about a pound a year (or 10 pounds a decade).

Over the last decade or so, the prevalence of obesity in the population of this country has increased alarmingly. The literature and the lay press have suggested many causes for this increase, ranging from the huge serving sizes of fast food, the high percentage of fat in the diet, and the ready availability of high-fructose corn syrup and other

sweeteners. Each of these “causes” may contribute, but no simple answer exists. The increased prevalence of obesity has been brought about by a combination of many physiological, psychological, and environmental factors. The interaction and contribution of all of the factors contribute to the overweight and obesity problem. The exact cause for the problem, or a way to solve it, is far from resolved. Though obesity clearly is a result of an energy imbalance, some have pointed out that obesity involves more than simply energy imbalance [59]. Besides those “causes” mentioned, other factors such as certain drugs, viruses, and toxins have been shown to be important in developing obesity in experimental animals. The importance of these factors has not been shown in humans, however.

This section covers some of the principles of regulating energy intake, storing excess energy, and losing body weight. Many of these regulatory factors have been discovered relatively recently, and the full story is not yet known. Because of the enormous scope of the problem and the economic effects on its health consequences, this is a very active area for research. This section prepares the reader to put the results of this new research into context as it is released.

GENETIC INFLUENCES

Our body shape and size has a strong genetic component. More than 127 candidate genes are associated with human obesity phenotypes. Every chromosome except the Y chromosome has one or more loci (the area on a chromosome that codes for a protein)[60]. A defect in some of these genes (such as those that involve the melanocortin receptor) can result directly in a person becoming obese. The melanocortin receptor is involved in the control of appetite and is covered later in this chapter.

Uncoupling proteins (covered in Chapter 5) also can play a role in developing obesity. Remember that uncoupling proteins are present in brown fat and uncouple phosphorylation (ATP synthesis) from electron transport. Polymorphisms have been shown to exist that produce alleles that result in greater weight gain in times of positive energy balance [60].

The heritability of body weight and composition also has been shown by studies of the body-weight changes in response to over-feeding or energy-restrictions feeding in pairs of identical twins. The individuals who shared the same genes responded in a similar manner, whereas some pairs of twins gained or lost weight more easily than others [61].

Awareness of the influence of genetics on body weight has led to a hypothesis that in general terms suggests that we each have a genetically predetermined body weight. If we go over that weight, we eat less (or exercise more), and if we go under that weight, we eat more. Obviously our environment can override the set point, or we would not be having an explosion in the prevalence of obesity. The large increase in the prevalence of obesity cannot be

explained by just genetics [61]. The set point has been one of the reasons used to explain the difficulty in successfully dieting and maintaining the weight loss for several years. Ninety-five percent of people who diet to lose weight gain back all or more within 5 years [59].

HORMONAL INFLUENCES

For the set-point theory to be valid, a mechanism must exist for controlling our food intake, both on a meal-to-meal basis and in the longer term. A recently identified hormone produced by the stomach and duodenum, called **ghrelin**, is an important hunger signal. Ghrelin secretion rises between meals when the stomach is empty. Ghrelin reacts with receptors in the hypothalamus and stimulates the release of neurotransmitters, such as neuropeptide Y and agouti-related protein. These neurotransmitters increase hunger. As a meal is consumed, ghrelin secretion rapidly diminishes, and hunger is reduced. [62,63].

The area of the hypothalamus that regulates hunger is called the **arcuate nucleus**. Two groups of neurons are produced in the arcuate nucleus. One group produces neurotransmitters of the melanocortin family, primarily melanocyte stimulating hormone (MSH). These neurons suppress hunger. MSH works by binding to a brain receptor. The other group of neurons in the arcuate nucleus produces neuropeptides Y and agouti-related protein. Agouti-related protein acts indirectly by inhibiting the appetite-suppressing actions of MSH.

An antagonistic hormone that regulates the urge to eat is cholecystokinin (CCK), produced in the intestine. CCK secretion rises during and after a meal and produces satiety (suppresses hunger). CCK is secreted following a meal to reduce appetite, which promotes satiety. A recently discovered hormone, secreted by the intestine, is called PYY; it suppresses the appetite for a longer time, about 12 hours. The amount of PYY secreted is proportional to the calorie content of the meal.

Also antagonistic to the hormones causing hunger is leptin, a hormone that interacts with the hypothalamus to reduce hunger. The polypeptide hormone **leptin** is secreted by adipose tissue. The more full the adipocyte, the more leptin it produces. Specific leptin receptors in the arcuate nucleus of the hypothalamus bind leptin to suppress the release of neuropeptides Y and to stimulate the release of MSH. MSH works to suppress hunger. The effect of leptin, then, is to control the level of stored body fat. As the level of body fat decreases, the amount of leptin secreted is reduced, and hunger is not suppressed as much. As the body fat level increases, the leptin level increases, and energy intake is reduced. The leptin mechanism has been theorized to be important in the plenty/famine cycles of our ancestors. This mechanism works well in normal-weight people. In overweight people, however, the receptors in the hypothalamus become defective, and increased

leptin levels do not suppress hunger [64]. For the interested reader see the article by Flier and Maratos-Flier listed under Suggested Readings.

Insulin is another hormone that suppresses hunger. Insulin stimulates the deposition of triacylglycerols in adipose tissue, which stimulates the release of leptin. Insulin is also thought to enter the brain and act directly on the leptin receptors of the hypothalamus to suppress the release of the neuropeptides Y and MSH. The exact role of insulin in regulating appetite is not well defined.

POSITIVE ENERGY BALANCE

As stated earlier in this chapter, weight changes are brought about by an imbalance in energy. Chapter 7 covered the integration of the metabolism of carbohydrate, protein, fat, and alcohol. The primary nutrients contributing to energy are alcohol (when present), carbohydrate, and fat in that order. When alcohol is consumed the liver converts it to two carbon units (acetate). The acetate is transported to the peripheral tissue, such as muscle, for oxidation. The acetate is not converted to fatty acids in the liver [65].

If alcohol is not involved, most of the body's energy comes from carbohydrate and fat. Carbohydrate (glucose) is first used for energy. When the body's energy needs are met, glucose is used to synthesize glycogen until these stores are filled. Only if additional glucose is present is it converted to fatty acids. If glucose is being used for energy, fewer fatty acids are being oxidized for energy. Remember the regulation of the TCA cycle, described in Chapter 3. If fatty acids are not being oxidized, fewer are needed, so less lipolysis takes place. Only when the quantity of carbohydrate exceeds the total energy needs does de novo synthesis of fatty acids occur. The newly synthesized fatty acids are made into triacylglycerols and are transported from the liver by VLDL. The fatty acids are taken up by the adipocyte. When sufficient carbohydrate is consumed to meet or exceed energy needs, very little fatty acids are oxidized for energy [65]. No tissue is solely dependent on fatty acids for energy, though heart muscle oxidizes fatty acids preferentially. Because the dietary fatty acids found in triacylglycerols are not used for energy, they are taken up by the adipocytes and stored as triacylglycerides without using very much metabolic energy. This fact is demonstrated by the fatty acid profile of storage fat, which resembles the fatty acid profile of the diet and not that of de novo synthesized fatty acids. The conversion of either glucose or amino acids to fatty acids is not efficient and requires considerable metabolic energy. Both glucose and amino acids must be converted first to acetyl CoA; then the acetyl CoA units are synthesized into fatty acids. Both steps require energy.

If the energy imbalance continues, the adipocytes in the body become enlarged. New adipocytes can be produced to accept the additional triacylglycerol. The number of fat cells increases most rapidly in late childhood and early puberty whenever a positive energy balance exists. This

process is called hyperplasia. Later in life, whenever the fat cells become enlarged (hypertrophy), their numbers can increase. Obese people have more, and larger, fat cells than normal-weight people. If body fat is lost, the number of fat cells does not decrease; they just get smaller.

NEGATIVE ENERGY BALANCE

Each year many people go on "diets" for the purpose of losing weight. Actually the goal is to lose adipose tissue and to retain lean body mass. Depending on the nature of the diet and the level of exercise both body fat and muscle may be lost. Little information is available on the proportion of fat and muscle lost on these diets. The goal of losing weight (body fat) over an extended period requires a negative energy balance. The most important part of a dietary intervention is for the calories consumed to be less than the calories expended. This ratio can be accomplished by either reducing energy intake, increasing energy expenditure through exercise, or a combination of these approaches. Controversy exists as to whether a difference in the proportions of macronutrients in a diet makes a difference in the amount of weight loss. Though agreement does not exist on this subject, in 1996 a study found that subjects in a hospital lost similar amounts of weight whether on a low- (15%) or high- (45%) carbohydrate diet [66]. The researchers provided 1000 kcal of energy. Significant decreases in total body fat and waist-to-hip circumferences occurred in both groups, but the size of these changes was not a function of diet. This finding suggests that the size of the caloric deficit and the length of time that a person is in negative energy balance have the greatest effect on the amount of weight lost. The popularity of weight loss diets with very low carbohydrate, high fat, and moderately high protein content has stimulated new research to determine if diets low in either carbohydrate or fat are more efficacious for weight loss. Several studies have shown that low carbohydrate diets with high fat and moderately high protein have resulted in greater weight loss over a six-month period. Most have shown that this advantage is lost after a year [66–72]. One study [71] matched subjects for energy and protein level in the diets and found no difference in the amount of weight lost. The researchers suggest that the amount of protein in the diet is the determining factor. In general, the risk factors for chronic disease (blood pressure and lipid profiles, etc.) are improved with weight loss, regardless of the diet used. This research is ongoing, with one study reporting results after one year of intervention [70]. More time and more research will be required before the issue is resolved. One article reviewed the literature on weight loss studies in obese adults and concluded that dietary/lifestyle therapy provides <5 kg weight loss after 2–4 years, pharmacologic therapy provides 5–15 kg weight loss after 1–2 years, and surgical therapy provides 25–75 kg weight loss after 2–4 years [73].

The low-carbohydrate, high-fat diets have been shown to bring about weight loss. Nutritional scientists do not

fully understand the mechanisms for this weight loss or know whether any long-term safety issues exist with these diets. Some studies have suggested that it is the satiety effects of the high-protein diets that cause the subjects to

consume less energy. Schoeller and associates [71] asks the question, “Does diet composition matter?” We will have to wait for further research to be reported before we can answer that question.

SUMMARY

Public health estimates of what we should weigh are based on body mass index. However, BMI does not evaluate body composition. With an accurate measure of height, weight, and skin folds from selected areas of the body, the percentage of body fat can be determined. Then, by difference, lean body mass can be estimated. Certain skin fold or circumference measurements, as well as use of more elaborate equipment such as DEXA and bioelectrical impedance analysis, can also provide some information about distribution of body fat, a factor that may be as important to health, or even more important to health than, percentage of total body fat.

Despite the differences in the various body components that have been noted among individuals and populations, the component that shows the greatest variability is clearly the one over which we have the most control—total body fat. Although changes in energy balance produce weight changes, the extent of these changes varies from person to person. One of the greatest problems in making predictions of energy needs centers around estimations of energy expenditure. Energy expenditure has three defined components: basal metabolic rate, thermic effect of foods, and the effect of exercise or physical activity, none of which is constant.

The prevalence of overweight and obesity has increased rapidly over the past 20 years and is now considered to be reaching epidemic proportions among children and adults. Morbidity and mortality from hypertension, stroke, coronary artery disease, dyslipidemia, type 2 diabetes, sleep apnea, and numerous other weight-related conditions are likely to increase because of the high prevalence of overweight and obesity in the U.S. population.

Our body shape and size has a strong genetic component. Defects in certain genes have been shown to cause obesity. For instance the defect in the gene that codes for the uncoupling protein can cause a change in our basal metabolism. The hypothalamic sites that control appetite are also genetically controlled. Appetite is regulated by opposing hormones such as ghrelin and CCK, which stimulate appetite, and leptin and insulin, which suppress hunger.

Each year many people go on a diet to lose weight. An active area of research is examining the efficacy and safety of energy-deficient diets that have different proportions of the macronutrients. At this time size of the caloric deficit appears to be more important to weight loss than the composition of the diet. Weight loss has been reported with both low-carbohydrate, high-fat diets and high-carbohydrate, low-fat diets.

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Suggested Reading

- Flier FS, Maratos-Flier E. What fuels fat. *Scientific American* 2007; 297:72–83

Eating Disorders

Despite its increasing prevalence in our society, obesity is still generally considered unacceptable. Few things can create as large a sensation in the media as a new weight reduction diet guaranteed to remove that unwanted fat. The authors of the sensational new diet are interviewed on television talk shows, newspapers give publicity to the new diet (and its authors), and the book promoting the new and revolutionary diet joins its companions on the shelves of all bookstores. The fact that the new diet book has so many companions on the bookshelves attests to the fact that none of these “new and revolutionary” diets is successful in helping people reduce weight and keep it off. Nevertheless, following some sort of weight reduction diet appears to be a way of life among many Americans, particularly women.

The desire by girls and women to be thin has a foundation: the ideal female body image is dictated to a large extent by movie and television celebrities, fashion models, and beauty pageant contestants. Society considers thin to be healthy. In fact, the body mass index of the winners of the Miss America pageant since 1922 has significantly declined over time, with recent winners considered undernourished, having body mass indices between 16.9 and 18.5 kg/m² [1]. Children as young as 9 years of age have been found curtailing their food intake to avoid becoming fat [2]. A female’s body size too often affects her self-worth and self-esteem. Body image distress results when people become dissatisfied with their weight, and, if people believe that their weight and shape are central to their self-worth as a person, an eating-disorder mind-set often ensues [3]. Eating disorders are particularly prevalent among young females. In fact, 90% to 95% of those affected by anorexia nervosa and bulimia are young, white females from middle- and upper-middle-class families; relatively few cases have been documented in males [4].

The incidence of eating disorders has consistently increased [5]. The prevalence of anorexia nervosa among U.S. adolescent girls and young women is estimated to be between 2% and 3% using the criteria of the American Psychiatric Association [6]. The estimated prevalence of bulimia nervosa is thought to be higher, ranging from 3% to 19% in adolescents and college-age females [7,8]. If the criteria of the American Psychiatric Association were not used, the prevalence of eating disorders or disordered eating likely would be much higher.

Anorexia Nervosa

Being too thin is dangerous, even deadly. Of the many psychiatric disorders, anorexia nervosa possesses the highest mortality rate [9]. If left untreated, up to

one-fifth of people with anorexia nervosa die within 20 years [10,11]. Given that this condition often affects children before they hit puberty, death may occur in persons only in their 20s or 30s.

Anorexia nervosa, described over 100 years ago as a loss of appetite caused by a morbid mental state, is actually misnamed, because its victims do not typically experience a loss of appetite. People with anorexia nervosa have a distorted body image and an irrational fear of weight gain. This distorted body image is a perception that they are fat even though they are extremely thin. Further, anorectics are extremely critical of their body as a whole and often more critical about selected body areas (such as thighs, stomach, etc.). Thus, they become obsessed with weight loss and relentlessly pursue thinness, often eating less than 800 kcal per day.

Eating patterns of people with anorexia nervosa mostly fall into one of two categories: the restricting type or the binge eating–purging type. Anorectics with the restricting type eat to a very limited extent without regularly inducing vomiting or misusing laxatives or diuretics. People with the binge eating–purging type alternate between restricting food intake and bouts of binge eating or purging behavior with laxative or diuretic misuse or self-induced vomiting [12,13]. However, in addition to these controlled eating behaviors, anorectics often exercise excessively to further weight loss efforts, to prevent possible weight gain, and to try to correct perceived imperfections in body size and shape. Exercise is considered excessive if its postponement is accompanied by intense guilt or when it is undertaken solely to influence weight or shape [14].

Women with anorexia nervosa often exhibit other personality traits, particularly perfectionism and obsessiveness. Obsessions related to orderliness and

symmetry are common [15]. In addition, they often have a poor self-image and want to please others because their perceived self-worth is heavily dependent on the words and actions of others (such as teachers, coaches, or instructors).

Diagnostic criteria for anorexia nervosa (Table 1) include refusal to maintain at least 85% of normal weight for height (or a body mass index of at least 17.5 kg/m²), denial of a low current body weight, fear of gaining weight (failure to show expected weight gain during growth), and amenorrhea (absence of at least three consecutive menstrual cycles) [16]. Preoccupation with food and abnormal food consumption patterns are also typical of anorexia nervosa. Changes in these criteria will likely occur with the publication of a fifth edition of the diagnostic manual [17].

The causes of anorexia nervosa are unknown, but the disease seems to be multifactorial. At least two sets of issues and behaviors are entangled. Issues include those concerning food and body weight and those involving relationships with oneself and with others [18–20]. Conflict regarding maturation and problems with separation, sexuality, self-esteem, and compulsivity often are associated with the development of anorexia nervosa [18]. A genetic basis for the condition also has been suggested.

The initial weight loss of the anorectic may not always be the result of a deliberate decision to diet; weight loss may occur unintentionally, for example, as the result of the flu or a gastrointestinal disorder [12]. However, following the initial weight loss, whatever its cause, additional diet restriction (and excessive exercise) is deliberate. Weight loss or control of body weight becomes the overriding goal in life, especially during stressful periods when pressures become overwhelming [12].

Table 1 Diagnostic Criteria for 307.1 Anorexia Nervosa

- A.** Refusal to maintain body weight at or above a minimally normal weight for age and height (e.g., weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).
- B.** Intense fear of gaining weight or becoming fat, even though underweight.
- C.** Disturbance in the way in which one’s body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight.
- D.** In postmenarcheal females, amenorrhea, i.e., the absence of at least three consecutive menstrual cycles. (A woman is considered to have amenorrhea if her periods occur only following hormone, e.g., estrogen, administration.)

Specify type:

Restricting Type: during the current episode of Anorexia Nervosa, the person has not regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

Binge-Eating/Purging Type: during the current episode of Anorexia Nervosa, the person has regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

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The anorectic learns the caloric contents of foods and the energy expenditure associated with various activities. Because anorectics have such a disturbed body image and such an intense fear of becoming fat, they may continue starving themselves to emaciation and even death should intervention be delayed too long.

The effects of anorexia nervosa on the body are similar to the effects of starvation (that is, protein-calorie malnutrition or **marasmus**) and affect all parts of the body [21,22]. Growth and development slow. Adipose tissue, lean body mass, and bone mass are lost. Organ mass may be lost, and organ function may become impaired. Loss of heart muscle can weaken the heart and cause an irregular heartbeat, among other serious complications. The gastrointestinal tract atrophies such that peristalsis is slowed, gastric emptying is delayed, and intestinal transit time is lengthened. The secretion of digestive enzymes and of digestive juices also is diminished. Constipation often results, along with abdominal distention after eating just small amounts of food. Hormone and nutrient levels in the blood become altered. Skin typically becomes dry, hair loss from the head occurs while **lanugo**-type (soft woolly) hair may appear on the sides of the face and arms, and body temperature drops. Table 2 lists some potential consequences of anorexia nervosa.

Treatment of anorexia nervosa is multidisciplinary (involving a physician, dietitian, nurse, psychologist, psychiatrist, and family therapist, among others) and may be accomplished through outpatient or inpatient care, depending on the severity of the person's condition. Assessment for inpatient treatment generally includes an evaluation of the person's mental status, how much the person is eating, current weight (inpatient treatment is warranted if weight is <25%–30% of ideal), speed of weight loss, motivation and adherence to treatment, family support, purging behavior, and comorbid complications, especially those affecting the heart [9,19]. Whether the patient is treated as an inpatient or as an outpatient, goals for the patient's health are established, often with a written contract signed by the patient as well as by members of the health care team.

Summaries of treatment outcomes involving thousands people treated for anorexia nervosa show that ~40% to 50% of them recover completely, ~30% improve, 20% to 25% continue to experience chronic problems with the condition, and another 10% to 15% die from medical complications, suicide, or malnutrition [7,23–25]. Mortality typically is highest

Table 2 Some Potential Medical Complications of Anorexia Nervosa

Gastrointestinal	Hematologic
Gastric distention	Anemia
Constipation	
	Skeletal
Cardiovascular	Stress fractures
Heart muscle atrophy	Premature osteoporosis
Bradycardia	
Hypotension	Muscle
Arrhythmias	Depleted muscle mass
Mitral valve prolapse	
Peripheral edema	Brain
	Abnormal electrical activity
Endocrine/metabolic	Confusion
Amenorrhea	
Low body temperature	

among people who have sustained severe weight loss, who have had the condition for a prolonged duration, and who developed the condition at an older age [6,7,19].

A long-term consequence of anorexia nervosa is osteopenia and ultimately osteoporosis, which occurs much earlier in those who have (or have had) anorexia nervosa than in those who have not suffered from the condition. Osteopenia and osteoporosis are associated with the amenorrhea and weight loss that occurs with anorexia nervosa. Factors associated with anorexia nervosa, such as poor energy and nutrient (especially vitamin D and calcium) intake, low blood estrogen concentrations, high blood cortisol concentrations, and low insulin-like growth factor 1 concentrations, contribute to the problem. Adolescents with anorexia nervosa are less likely to reach peak bone mass. Bone formation is impaired and bone resorption increased in adolescents and women with anorexia nervosa, leading to diminished bone mineral density, premature osteoporosis, and increased fracture risk [26,27]. Bone mineral density has been shown to be significantly correlated with the age of onset and the duration of anorexia nervosa [27]. Although weight gain and the resumption of normal menstrual function can improve bone mineral density, the bones do not appear to catch up to normal to correct the deficit that occurred during the amenorrhea [28,29]. Studies investigating the effectiveness of hormone replacement and other drugs for the treatment of osteoporosis associated with anorexia nervosa in young women are ongoing.

Bulimia Nervosa

Bulimia nervosa, another eating disorder, is a condition characterized by recurring binge eating coupled with self-induced vomiting and misuse of laxatives, diuretics, or other medications to prevent weight gain. Binge eating is marked by a sense of lack of control over eating during the binge episode. A binge is defined as eating an amount of food larger than most people would eat during a similar time period and under similar circumstances [16]. Bulimia denotes a ravenous appetite (or "ox hunger") associated with powerlessness to control eating [12]. Criteria [16] for the diagnosis of bulimia nervosa are given in Table 3.

Bulimia occurs primarily in young women, especially college-age women who are of normal weight or slightly overweight. The typical bulimic, rather than being overly concerned with losing weight and becoming very thin (like the person with anorexia nervosa), seeks to be able to eat without gaining weight [12]. Other factors associated with the development of bulimia include a history of sexual abuse, psychoactive substance abuse or dependence, and a family history of depression or alcoholism [7,30].

Bulimia often starts with dieting attempts in which hunger feelings get out of control. These dieting attempts, usually based on food abstinence or excessive food restriction, lead to binge eating. Once binge eaters discover that they can undo the consequences of their overeating by vomiting the ingested food, they begin to binge not only when they are hungry but also when they are experiencing any distressing emotion [7,12].

Table 3 Diagnostic Criteria for 307.51 Bulimia Nervosa

- A. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:
- (1) eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances
- (2) a sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating)
- B. Recurrent inappropriate compensatory behavior in order to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.
- C. The binge eating and inappropriate compensatory behaviors both occur, on average, at least twice a week for 3 months.
- D. Self-evaluation is unduly influenced by body shape and weight.
- E. The disturbance does not occur exclusively during episodes of Anorexia Nervosa.

Specify type:

Purgung Type: during the current episode of Bulimia Nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

Nonpurgung Type: during the current episode of Bulimia Nervosa, the person has used other inappropriate compensatory behaviors, such as fasting or excessive exercise, but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

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Most binge eating is done privately in the afternoon or evening, with an intake of about 3,500 kcal [31,32]. Favorite foods for binging usually are dessert and snack foods very high in carbohydrates. Embarrassment usually prevents the bulimic from revealing his or her food-related behavior even to those closest to him or her [4].

Diagnosis is usually dependent on self-reported symptoms or on treatment for related problems or conditions. Conditions that may develop as the result of bulimia are listed in Table 4. The gastrointestinal tract is greatly affected by repeating vomiting and the use of laxatives. Repeated vomiting also causes other problems, including skin lesions or calluses on the dorsal side of the hands (especially over the joints), severe dental erosion, swollen enlarged neck glands, reddened

eyes, headache, and fluid and electrolyte imbalances. Laxative misuse may exacerbate fluid and electrolyte losses and, when coupled with vomiting, may lead to heart arrhythmias and heart failure. The presence of lesions or calluses on the hands, the swollen neck glands, and the frequent trips to the bathroom after meals often are recognized by health professionals and family or friends and facilitate detection and diagnosis of the problem.

The treatment of bulimia, like that of anorexia nervosa, is multidisciplinary. Goals typically focus on eliminating binge-purge behaviors, normalizing eating habits, maintaining weight, and resuming normal menses, if they are affected [7]. The patient is most likely to be hospitalized with problems such as electrolyte imbalance, drug (e.g., laxatives, diuretics)

dependence, severe depression, or suicidal tendencies [7]. The prognosis of those suffering from bulimia nervosa is similar to those with anorexia nervosa. About 50% of people recover, but about half of these continue to have some abnormal eating habits; another 30% continue to have a nonspecified eating disorder [7].

Binge Eating Disorder

The American Psychiatric Association *Diagnostic Manual of Mental Disorders* includes a provisional eating disorder diagnosis, Binge Eating Disorder, which is not associated with purging as in bulimia nervosa and which requires further study [16]. Binge eating disorder is characterized by binge eating at least twice a week for at least a 6-month period with no compensatory behaviors. The binge eating episode typically involves eating (usually with a general sense of lack of control) large amounts of high energy dense foods (such as dessert and snack food type items) more rapidly than normal, and continues despite feeling uncomfortably full. Subtypes of the disorder are mainly distinguished by whether the binge precedes dieting or whether dieting precedes the binge eating [33].

For those with a binge eating disorder, food often provides comfort and a sense of emotional well-being, especially if the person is feeling stressed, anxious, unhappy, or depressed [34,35]. Thus, the binge usually occurs when the person is not physically hungry, but is emotionally unhappy. The binge usually is done in private because of embarrassment. Moreover, following the binge, the person is often disgusted with herself or feels depressed or guilty.

Disordered Eating

Eating disorders (Table 5) [16] other than anorexia nervosa, bulimia nervosa, and binge eating disorder also are present within the U.S. population and are categorized by the American Psychiatric Association as Eating Disorders Not Otherwise Specified. The characteristics of those with disordered eating are similar to those of anorexia nervosa and bulimia and include fear of being fat, restrained eating, binge eating, purge behavior, and distorted body image; however, people with disordered eating do not meet the criteria for anorexia nervosa or bulimia nervosa (Tables 1 and 3) [36]. Disordered eating is common among female athletes and often exists as part of what is known as the female athlete triad.

Table 4 Some Potential Medical Complications of Bulimia Nervosa

Gastrointestinal	Cardiovascular
Erosion of the teeth	Arrhythmias
Dental caries	
Sore throat	Respiratory and skeletal
Swollen parotid glands	Aspiration pneumonia
Esophageal rupture or tears	Rib fracture
Stomach tear	
Gastroesophageal reflux disease	Endocrine/metabolic
Constipation	Irregular menses or amenorrhea
Cathartic colon	Electrolyte imbalance

Table 5 307.50 Eating Disorder Not Otherwise Specified

The Eating Disorder Not Otherwise Specified category is for disorders of eating that do not meet the criteria for any specific Eating Disorder. Examples include

1. For females, all of the criteria for Anorexia Nervosa are met except that the individual has regular menses.
2. All of the criteria for Anorexia Nervosa are met except that, despite significant weight loss, the individual's current weight is in the normal range.
3. All of the criteria for Bulimia Nervosa are met except that the binge eating and inappropriate compensatory mechanisms occur at a frequency of less than twice a week or for a duration of less than 3 months.
4. The regular use of inappropriate compensatory behavior by an individual of normal body weight after eating small amounts of food (e.g., self-induced vomiting after the consumption of two cookies).
5. Repeatedly chewing and spitting out, but not swallowing, large amounts of food.
6. Binge-eating disorder: recurrent episodes of binge eating in the absence of the regular use of inappropriate compensatory behaviors characteristic of Bulimia Nervosa.

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The Female Athlete Triad

The female athlete triad, defined as the combination of disordered eating, amenorrhea, and osteopenia, was first described in 1992 [37]. The condition appears most often in women participating in sports in which physique and body image are important and extra body weight is undesirable. Women most at risk include long-distance runners, figure skaters, gymnasts, ballet dancers, and swimmers and divers [7,28,38]. However, because of societal pressures to be thin and because of the perfectionist tendencies of many females, all female athletes are at risk for the female athlete triad [39].

Disordered eating among athletes is common, with an estimated prevalence of 15% to 62%. The disordered eating, however, can vary considerably. To lose or maintain body weight, some athletes may restrict food intake mildly to severely while others may also use diet pills, diuretics, and laxatives. Still others may binge with or without purging.

Whereas amenorrhea occurs in only 2% to 5% of women in the general population, 3% to 66% of female athletes experience amenorrhea [28]. The amenorrhea that occurs in athletes may be primary or secondary. Generally, primary amenorrhea is found in females who by about age 14 years have not menstruated. Secondary amenorrhea refers to the absence of three to six menstrual cycles in a year in females who have previously had a regular menstrual cycle or to a 1-year absence of menstrual bleeding in females with previously irregular periods [28,40]. The causes of amenorrhea in athletes, as in women with eating disorders, are not clearly understood. They are thought to relate to synergistic effects of excessive amounts of physical activity and training, constant stress or anxiety, low amounts of body fat, weight fluctuations, and

poor diet, especially an extreme energy (caloric) deficit [40,41]. The effect of these various factors is to evoke changes in the release of hormones such as follicular stimulating and luteinizing hormones that then lead to alterations in the release of estrogen and progesterone (among other hormones), which in turn can cause amenorrhea. The amenorrhea—more specifically, the diminished serum estrogen concentrations—in turn causes medical problems.

One main complication of an estrogen deficit is its effect on the body's skeletal system. Female athletes with amenorrhea and disordered eating have an increased risk for osteopenia and ultimately osteoporosis. The young athletes exhibit premature bone loss, inadequate bone formation, or both with resulting low bone mass, stress fractures, and other orthopedic problems [41,42]. High levels of hormones such as cortisol, common in athletes, and extensive training regimens also can contribute to bone loss. Although weight-bearing exercises promote bone mineralization to improve bone density, this protection is considerably diminished without estrogen [38]. Young athletes lose bone during a phase of life when they should be accruing bone, and thus they compromise the attainment of peak bone mass. Studies have found that over 50% of athletes with amenorrhea have low bone mass or bone densities at least one standard deviation below the mean [7,43,44]. As with others experiencing eating disorders, although resuming menses helps improve bone mass, recovery from the loss of bone associated with amenorrhea is thought to be incomplete [45–47].

The early identification and treatment of eating disorders is crucial if serious complications are to be avoided. Just as obesity is associated with increased risk for a variety of diseases, eating disorders are associated

with a multitude of risks, including death. Combating eating disorders is difficult. Not only must the victims be treated, but the images and values of society must also be rehabilitated [48].

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Web Sites

- www.anred.com
www.nationaleatingdisorders.org
www.nimh.nih.gov/publicat/eatingdisorders.cfm

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9

The Water-Soluble Vitamins

Vitamin C (Ascorbic Acid)

Thiamin (Vitamin B₁)

Riboflavin (Vitamin B₂)

Niacin (Vitamin B₃)

Pantothenic Acid

Biotin

Folate

Vitamin B₁₂ (Cobalamin)

Vitamin B₆

For each vitamin, the following subtopics (when applicable) are discussed:

Sources

Digestion, Absorption, Transport, and Storage

Functions and Mechanisms of Action

Interactions with Other Nutrients

Metabolism and Excretion

Recommended Dietary Allowance or Adequate Intake

Deficiency

Toxicity

Assessment of Nutriture

PERSPECTIVE

Genetics and Nutrition: The Possible Effect on Folate Needs and Risk of Chronic Disease

The early part of the 20th century marks the most exciting era in the history of nutrition science. It was during this time that the discovery of vitamins, or “accessory growth factors,” began. Researchers found that for life and growth, animals required something more than a chemically defined diet consisting of purified carbohydrate, protein, fat, minerals, and water. The first of these dietary essentials to be discovered was an antiberiberi substance isolated from rice polishings by Casimir Funk, a Polish biochemist. Funk gave it the name *vitamine* because the substance was an amine and necessary for life. Very shortly thereafter McCollum and Davis extracted a factor from butter fat that they called fat-soluble A to distinguish it from the water-soluble antiberiberi substance. These two essential factors became known as vitamine A and vitamine B.

As each additional vitamin was discovered, it was assigned a letter. The *e* on *vitamine* was dropped to give the general name *vitamin*, because only a few of the essential substances were found to be amines. As the chemical structure of a vitamin became known through its isolation and synthesis, it was given a chemical name. Each chemical name assigned was assumed to apply only to one substance, with one specific activity. We now know that a vitamin may have a variety of functions, and that vitamin activity may be found in several closely related compounds, known as vitamers. An excellent example of this range of activity is vitamin A, which has several seemingly unrelated functions and encompasses not only retinol but also retinal and retinoic acid.

Vitamins are organic compounds with regulatory functions that are required in the diet if the species (humans) is unable to synthesize them. Thus, vitamins are considered essential (in fact *vita* means “life” in Latin). Moreover, because these substances must be supplied by the diet, their discovery often came about because of their absence in the diet. Although in the case of a deficiency, the clinician should be able to recognize the syndrome caused by a lack of the particular vitamin, in this country of abundant and varied food supply the nutrition professional should instead think in terms of what a specific vitamin does rather than what disease it prevents. Unfortunately, relating the function of the vitamin directly to its deficiency syndrome is often impossible.

Vitamins, for the most part, are not related chemically and differ in their physiological roles. The broad classifications of water-soluble vitamins and fat-soluble vitamins are made because of certain properties common to each group. The fat-soluble vitamins are discussed in Chapter 10. The body handles the water-soluble vitamins differently from the way it handles the fat-soluble vitamins. They are absorbed into portal blood, in contrast to fat-soluble vitamins, and, with the exception of cobalamin (vitamin B₁₂), they cannot be retained for long periods by the body. Any storage that occurs results from their binding to enzymes and transport proteins. Water-soluble vitamins are excreted in the urine whenever plasma levels exceed renal thresholds.

With the exception of vitamin C (ascorbic acid), water-soluble vitamins are members of the B complex. Most of the B-complex group can be further divided according to general function: energy releasing or hematopoietic. Other vitamins cannot be classified this narrowly because of their wide range of functions. Figure 9.1 shows the classification of water-soluble vitamins.

In this chapter, discussions of the various vitamins are grouped similarly. Each vitamin is considered (when precise information is available) in terms of structure, sources, absorption (also digestion where applicable), transport, storage, functions and mechanisms of action, metabolism and excretion, recommended dietary allowance or adequate intake, deficiency, toxicity, and assessment of nutriture. Specific interrelationships with other nutrients are also noted for selected vitamins. Table 9.1 contains a summary of the coenzyme form, functions, deficiency

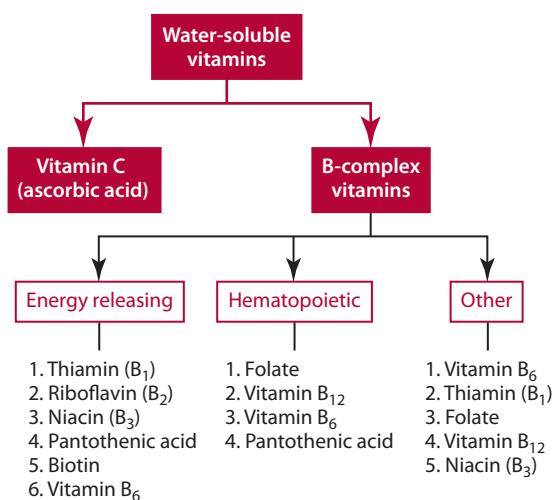


Figure 9.1 The water-soluble vitamins.

Table 9.1 The Water-Soluble Vitamins: Functions, Deficiency Syndromes, Food Sources, Recommended Intake, and Individuals at Risk for Deficiency

Water-Soluble Vitamins						
Vitamin	Main Coenzymes	Biochemical or Physiological Function	Deficiency Syndrome or Symptoms	Good Food Sources	RDA* or AI†	Some Conditions and/or Individuals at Risk for Deficiency
Thiamin (vitamin B ₁)	Thiamin diphosphate (TDP) or thiamin pyrophosphate (TPP)	Oxidative decarboxylation of α-keto acids and 2-keto sugars	<i>Beriberi</i> , muscle weakness, anorexia, tachycardia, enlarged heart, edema	Yeast, pork, sunflower seeds, legumes	1.1 mg* 1.2 mg	Alcoholism, elderly, malabsorptive conditions
Riboflavin (vitamin B ₂)	Flavin adenine dinucleotide (FAD); flavin mononucleotide (FMN)	Electron (hydrogen) transfer reactions	<i>Ariboflavinosis</i> , cheilosis, glossitis, hyperemia and edema of pharyngeal and oral mucous membranes, angular stomatitis, photophobia	Beef liver, braunschweiger sausage, steak, mushrooms, ricotta cheese, nonfat milk, oysters	1.1 mg* 1.3 mg	Alcoholism, heart failure, hypermetabolic conditions
Niacin (vitamin B ₃) (nicotinic acid, nicotinamide)	Nicotinamide adenine dinucleotide (NAD); nicotinamide adenine dinucleotide phosphate (NADP)	Electron (hydrogen) transfer reactions	<i>Pellagra</i> , diarrhea, dermatitis, mental confusion or dementia	Tuna, beef liver, chicken breast, beef, halibut, mushrooms	14 mg* 16 mg	Alcoholism, malabsorptive conditions, Hartnup disease
Pantothenic acid	Coenzyme A (CoA)	Acyl transfer reactions	Deficiency very rare; numbness and tingling of hands and feet, vomiting, fatigue	Widespread in foods; exceptionally high amounts in egg yolk, liver, kidney, yeast	5 mg†	Alcoholism, malabsorptive conditions
Biotin	N-carboxybiotinyl lysine	CO ₂ transfer/ carboxylation reactions	Deficiency very rare, anorexia, nausea, glossitis, depression, dry scaly dermatitis	Synthesized by microflora of digestive tract; yeast, liver	30 µg†	Excessive raw egg ingestion, alcoholism, malabsorptive conditions
Vitamin B ₆ (pyridoxine, pyridoxal, pyridoxamine)	Pyridoxal phosphate (PLP)	Transamination and decarboxylation reactions	Dermatitis, glossitis, convulsions	Steak, navy beans, potato, salmon, banana, whole grains	1.3 mg*	Elderly, alcoholism, use of certain medications

(Continued)

Table 9.1 Continued

Water-Soluble Vitamins						
Vitamin	Main Coenzymes	Biochemical or Physiological Function	Deficiency Syndrome or Symptoms	Good Food Sources	RDA* or AI†	Some Conditions and/or Individuals at Risk for Deficiency
Folate	Derivatives of tetrahydrofolic acid: 5,10-methylene THF, 10-formyl THF, 5-formimino THF, 5,10-methylencyl THF, 5-methyl THF	One-carbon transfer reactions	<i>Megaloblastic anemia, diarrhea, fatigue, depression, confusion</i>	Brewer's yeast, spinach, asparagus, turnip greens, lima beans, beef liver	400 µg*	Alcoholism, malabsorptive conditions, use of certain medications
Vitamin B ₁₂ (cobalamin)	Methyl cobalamin, adenosyl cobalamin	Methylation of homocysteine to methionine; conversion of methylmalonyl CoA to succinyl CoA	<i>Megaloblastic anemia, degeneration of peripheral nerves, skin hypersensitivity, glossitis</i>	Meat, fish, shellfish, poultry, milk	2.4 µg*	Elderly, strict vegetarians, pernicious anemia, some disorders affecting stomach and ileum
Ascorbic acid (vitamin C)	None	Antioxidant, cofactor of hydroxylating enzymes involved in synthesis of collagen, carnitine, norepinephrine	<i>Scurvy, fatigue, retarded wound healing, bleeding gums, spontaneous rupture of capillaries</i>	Papaya, orange juice, cantaloupe, broccoli, brussels sprouts, green peppers, grapefruit juice, strawberries	75 mg* 90 mg	Elderly, alcoholism, smoking

*Adults age 19 to 50 years, females and males respectively.

†Adequate intake.

syndrome, those at risk for deficiency, sources, and recommended dietary allowance (RDA) or adequate intake (AI) of each of the water-soluble vitamins. The inside book covers provide the dietary reference intakes (DRIs), when available, for all nutrients and for all age groups.

DRIs represent quantitative approximations of nutrient intakes for the purpose of planning and assessing the diets of healthy people. DRIs include RDAs as well as AIs, tolerable upper intake levels (ULs), and **estimated average requirements (EARs)**. RDAs represent the average daily dietary intake level that is sufficient to meet the nutrient requirements of about 97% of healthy people. They are based on EARs, which are the amounts of nutrients thought to meet the nutrient requirements of 50% of the healthy people in a specified age and gender group. RDAs are set higher than EARs by either two standard deviations or a coefficient of variation for the EAR. Thus, nutrient intakes are likely inadequate if intake is significantly less than the EAR. Further, if intakes are above the EAR but less than the RDA, it may still be inadequate. AIs are provided for nutrients instead of RDAs when scientific data are insufficient to calculate the EAR for the given nutrients. AIs are based on nutrient intake levels of healthy people (with adequate nutritional status), and are typically thought to exceed the requirement for the nutrient. Thus, nutrient intakes are likely adequate if they equal or exceed the AI, but may or may not be adequate if they are less than the AI. If nutrient intakes are above the RDA but less than the UL, then they are likely to be adequate. ULs provide the highest intake level for a

nutrient that is unlikely to cause any risk of adverse health to almost all people in the age- or gender-specified groups. ULs are viewed as maximum amounts for those consuming fortified foods or supplements in large quantities. For some nutrients, the UL is not known, but the lack of an UL does not mean that large doses of the nutrient is without harm.

Suggested Reading

McCollum EV. A History of Nutrition. Boston: Houghton Mifflin, 1957.

Vitamin C (Ascorbic Acid)

The human being is one of the few mammals unable to synthesize vitamin C, also known as ascorbic acid or ascorbate. Other animals unable to synthesize vitamin C include primates, fruit bats, guinea pigs, and some birds. The inability to synthesize vitamin C results from the lack of gulonolactone oxidase, the last enzyme in the vitamin C synthetic pathway. The synthetic pathway and structure of the vitamin are shown in Figure 9.2, which indicates that vitamin C is a six-carbon compound. The L-isomer of the vitamin is the one that is biologically active in humans.

Vitamin C was isolated in 1928, and its structure was determined in 1933, but the problems referred to as scurvy and associated with the lack of vitamin C had been quite prevalent for centuries. Some of the most notable stories are those of the British sailors who often died from scurvy on sea

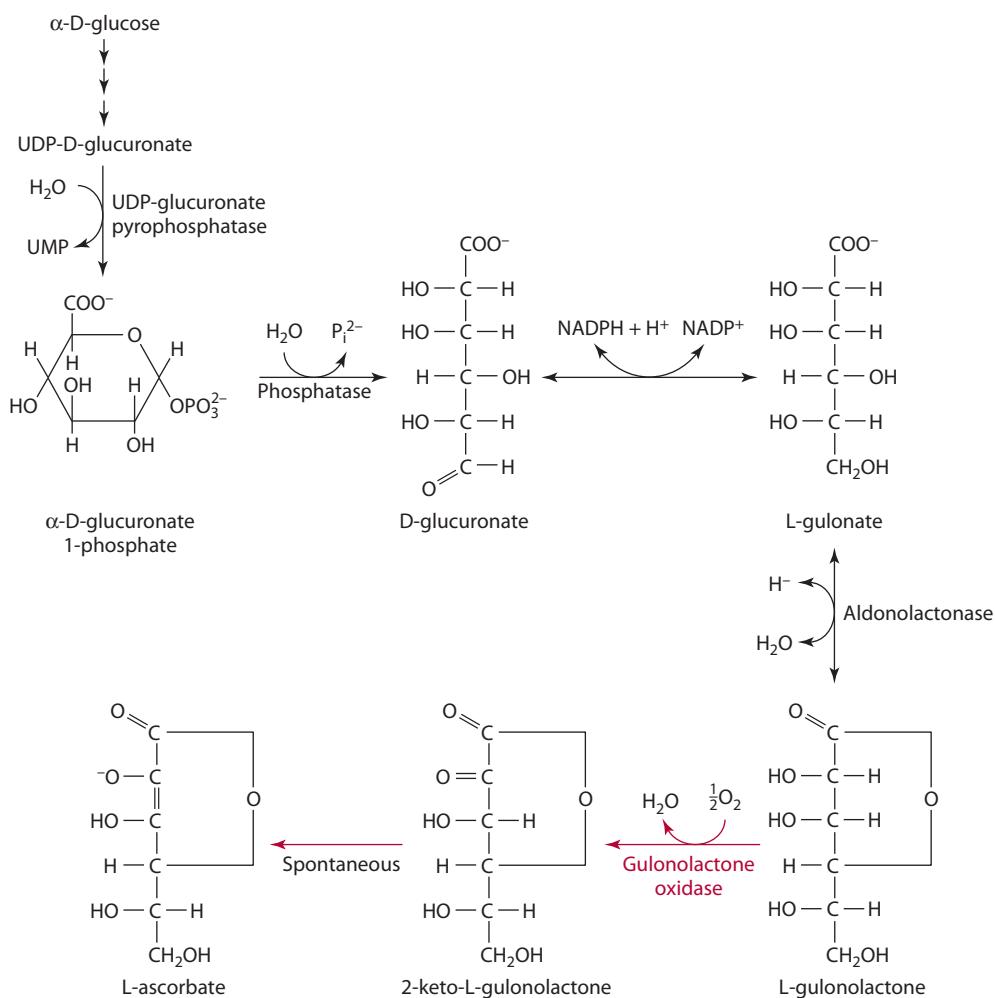


Figure 9.2 Synthesis of ascorbic acid. Humans lack the gulonolactone oxidase that catalyzes the final enzymatic reaction.

voyages. It was in the late 1790s and early 1800s that British sailors at sea began receiving limes (resulting in the nickname Limey for the sailors) in an effort to prevent the outbreaks; the use of citrus as a cure for scurvy was shown back in 1753. Szent-Györgyi (1928) and King (1932) are considered co-discoverers of vitamin C. Szent-Györgyi, who isolated the vitamin, and Haworth, who determined its structure, were awarded the Nobel prize in 1937 for their vitamin C work.

SOURCES

The best food sources of vitamin C include asparagus, papaya, oranges, orange juice, cantaloupe, cauliflower, broccoli, Brussels sprouts, green peppers, grapefruit, grapefruit juice, kale, lemons, and strawberries. Of these foods, citrus products are most commonly cited as significant sources of the vitamin. Supplements supply vitamin C typically as free ascorbic acid, calcium ascorbate, sodium ascorbate, and ascorbyl palmitate. Rose hip (*Rosa*), a seed capsule found in roses, also contains vitamin C and is used commercially in vitamin C supplements; vitamin C from rose

hips does not appear to be superior to other vitamin C sources such as orange juice.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Vitamin C does not require digestion prior to being absorbed into intestinal cells. Absorption of ascorbate (but not dehydroascorbate) across the brush border occurs throughout the small intestine, including the ileum, by at least two different sodium-dependent cotransporters, designated SVCT1 and SVCT2 [1]. SVCT1 appears to have a higher capacity for ascorbate than SVCT2 [2]. Sodium-dependent transporters also are thought to be responsible for vitamin C uptake into most organs. SVCT1 is found in most epithelial tissues. SVCT2 is also present in most tissues except skeletal muscle and lungs. Simple diffusion, which may occur throughout the stomach and small intestine, provides for vitamin C absorption with ingestion of higher amounts of the vitamin. Anion channels in some cells may mediate vitamin C diffusion faster than the transporters.

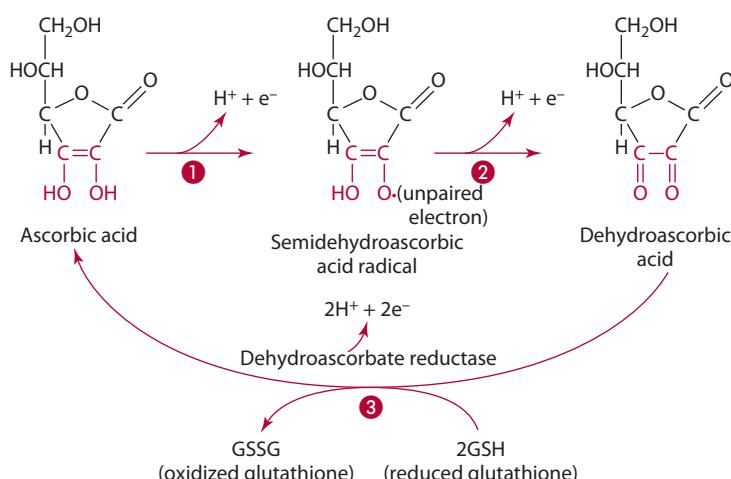
Prior to absorption, ascorbate may be oxidized (two electrons and two protons removed) to form dehydroascorbate (Figure 9.3), which may be absorbed by facilitated diffusion using sodium-independent carriers [2]. In addition, dehydroascorbate (but not ascorbate) competes with glucose for uptake by glucose (GLUT) transporters, especially GLUT 1 and GLUT 3 [2]. Absorption of dehydroascorbate is thought to occur to a greater extent than absorption of ascorbate [2,3]. Yet within the intestinal cells (but also other cells) dehydroascorbic acid is rapidly reduced back (recycled) to ascorbic acid (Figure 9.3) by the enzyme dehydroascorbate reductase, which exhibits reductase activity. Glutathione (GSH), which is required for the reduction of dehydroascorbate, is oxidized (GSSG) in the process as shown in Figure 9.3. Glutathione spares vitamin C and, in general, improves the antioxidant protection capacity of blood [4,5]. NADPH and glutaredoxin (a dithiol) also can be used to reduce the dehydroascorbate.

Notice in Figure 9.3 that during the oxidation of ascorbate, a free radical called semidehydroascorbic acid radical (also called ascorbate free radical, ascorbyl, monodehydroascorbate radical, or a 1-electron oxidation product) is formed. The ascorbate free radical is thought to have a short half-life and reacts poorly with oxygen (and thus does not typically generate reactive oxygen species such as superoxides). Instead, the ascorbate free radical either is oxidized to dehydroascorbate or reacts with another semidehydroascorbate radical to form ascorbate and dehydroascorbate (2 semidehydroascorbate radicals → Ascorbate + dehydroascorbate). Other reactions that help to regenerate ascorbate from dehydroascorbate and from semidehydroascorbate are shown under “Antioxidant Activity” in the Functions and Mechanisms of Action section of vitamin C.

The degree of vitamin C absorption decreases with increased vitamin intake. Absorption can vary from 16% at high intakes (~12 g) to 98% at low intakes (<20 mg) [6]. Over a range of usual intakes (30–180 mg/day) from food, the average overall absorption is about 70% to 95% [7,8]. Absorption of ascorbate may be diminished in the presence of high intracellular glucose, which appears to interfere with the ascorbate transporter [2,9]. From the intestinal cells, ascorbate diffuses through anion channels into extracellular fluid and enters the plasma by way of capillaries [2]. Unabsorbed vitamin C may be metabolized by intestinal flora. Ingesting large amounts of iron with vitamin C may result in the oxidative destruction of the vitamin in the digestive tract, yielding diketogulonic acid and other products without vitamin C activity [7].

Absorbed ascorbic acid is transported in the plasma primarily in free form, as an ascorbate anion [2,10]. Normal plasma concentrations of ascorbate range from about 0.4 to 1.7 mg/dL; higher plasma concentrations can be achieved with intravenous administration of the vitamin than with oral [11]. Uptake of ascorbate into body cells requires sodium and a carrier, and into some cells, such as leukocytes (also called white blood cells), uptake is also energy dependent. Tissue concentrations of vitamin C usually exceed plasma concentrations, with the magnitude dependent on the specific tissue. Cells usually become saturated before plasma. The vitamin C content of white blood cells, for example, can be as high as 80 times greater than plasma concentrations. Only small amounts of dehydroascorbate appear in the blood, because of rapid cellular uptake by GLUT transporters [2,10].

Ascorbate and dehydroascorbate concentrations are much greater in some tissues than in others. The highest concentrations of vitamin C are found in the adrenal and



- ① During the oxidation of ascorbic acid, a free radical called semidehydroascorbic acid radical is formed but has a short half-life.
- ② Oxidation of the radical forms dehydroascorbic acid.
- ③ Dehydroascorbic acid can be reduced with hydrogens provided by the reduced form of glutathione.

Figure 9.3 The interconversion of ascorbate and dehydroascorbate.

pituitary glands (with each possessing ~30–50 mg/100 g of wet tissue) [12]. Intermediate levels of vitamin C are found in the liver, spleen, heart, kidneys, lungs, pancreas, and leukocytes, and smaller amounts occur in the muscles and red blood cells [12]. In absolute terms based on total weight, the liver contains the most vitamin C [2]. The maximal vitamin C pool is estimated at about 1,500 mg [8]. Intakes of about 100 to 200 mg vitamin C per day have been shown to produce plasma concentrations of about 1.0 mg/dL and to maximize the body pool [8].

FUNCTIONS AND MECHANISMS OF ACTION

Despite its uncomplicated structure, vitamin C has very complex functional roles in the body. Ascorbic acid is required in several reactions involved in body processes, including collagen synthesis, carnitine synthesis, tyrosine synthesis and catabolism, and neurotransmitter synthesis. In these reactions, vitamin C functions as a reducing agent (antioxidant) to maintain the iron and copper atoms in the metalloenzymes in the reduced state. In addition to its role as a reducing agent in enzymatic reactions, vitamin C functions in other capacities as an important antioxidant in the body. Each of these processes, as well as some additional roles of vitamin C, is reviewed in this section.

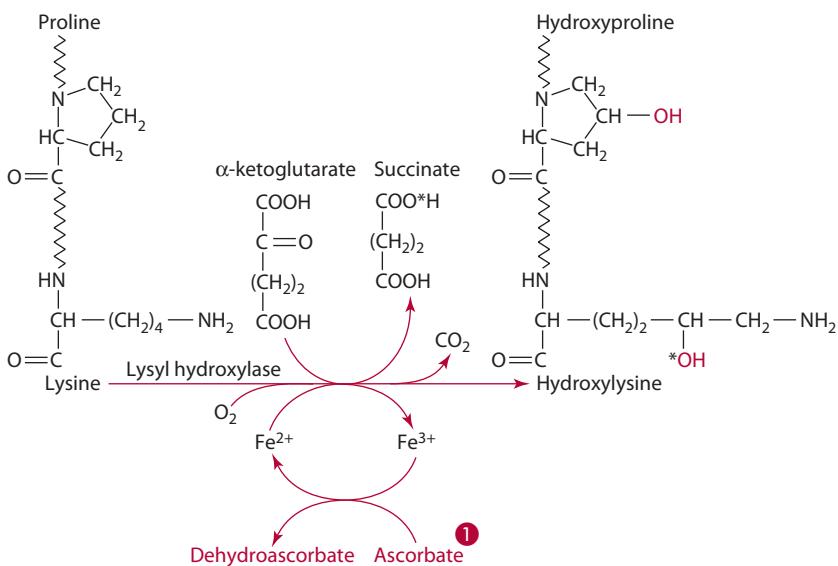
Collagen Synthesis

Ascorbate functions in a number of hydroxylation reactions. Three hydroxylation reactions requiring vitamin C are necessary for collagen synthesis. Remember that collagen is a structural protein found in skin, bones, tendons, and cartilage. After the collagen chains are made, the vitamin C-dependent hydroxylation reactions

occur posttranslationally. These reactions are important in order for the collagen molecule to aggregate and cross-link into its triple-helix configuration. Prolyl 4-hydroxylase and prolyl 3-hydroxylase (also called dioxygenases) catalyze the hydroxylations of specific proline residues on newly synthesized collagen α chains. Proline and hydroxyproline provide more rigidity to the collagen. Lysine hydroxylation by lysyl hydroxylase (also called dioxygenase) results in the formation of hydroxylysyl residues. These hydroxylysyl residues may undergo additional posttranslational modifications, such as glycosylation and phosphorylation. The lysine and hydroxylysyl residues also are further acted upon by lysyl oxidase, a copper-dependent enzyme that facilitates cross-linking between collagen molecules to provide added strength.

The role of vitamin C in the hydroxylation reactions relates to the iron cofactor. Prolyl hydroxylases and lysyl hydroxylase both require iron bound as a cofactor. During the reactions, the dioxygenases catalyze reactions in which one of two atoms of O_2 becomes incorporated into the product, and the second of the two atoms of O_2 becomes incorporated into the cosubstrate α -ketoglutarate to form the new carboxyl group of succinate (Figure 9.4). During the hydroxylation reactions, the iron cofactor in the enzymes is oxidized; that is, it is converted from a ferrous ($2+$) state to a ferric ($3+$) state. Ascorbate is needed to function as the reductant, thereby reducing iron back to its ferrous state ($2+$) in the prolyl and lysyl hydroxylases.

Although these reactions may seem simple, normal development and maintenance of skin, tendons, cartilage, bone, and dentine depend on an adequate supply of vitamin C. Also, the basement membrane lining the capillaries, the “intracellular cement” holding together the endothelial cells, and the



① Ascorbate acts as a reducing agent to convert the oxidized iron atom (Fe^{3+}) back to its reduced state (Fe^{2+}) in the enzymes lysyl hydroxylase and prolyl hydroxylase.

Figure 9.4 Ascorbate functions in the hydroxylation of peptide-bound proline and lysine in procollagen. One atom of oxygen (*) appears in the hydroxyl group of the product and the other in succinate.

scar tissue responsible for wound healing all require the presence of vitamin C for their formation and maintenance.

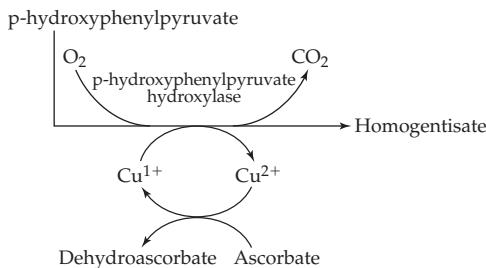
Carnitine Synthesis

Ascorbate is involved in two reactions required for the synthesis of carnitine. Remember, carnitine is a non-protein, nitrogen-containing compound made from the amino acid lysine, which has been methylated using S-adenosyl methionine (SAM). Producing sufficient carnitine is critical in fat metabolism, because carnitine is essential to transport long-chain fatty acids from the cell cytoplasm into the mitochondrial matrix where β -oxidation occurs. The reactions in carnitine synthesis (Figure 6.12) involving ascorbate are hydroxylations similar to those for proline and lysine hydroxylation [13]. Vitamin C functions as the preferred reducing agent, specifically reducing the iron atom from the ferric state (Fe^{3+}) back to the ferrous state (Fe^{2+}).

Tyrosine Synthesis and Catabolism

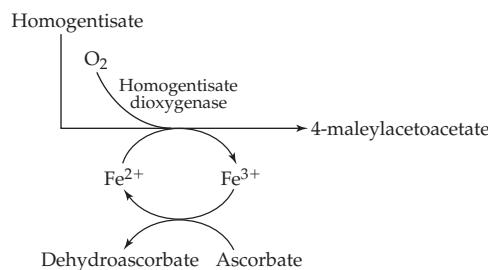
Tyrosine is synthesized in the body from the essential amino acid phenylalanine. Tyrosine synthesis requires hydroxylation of phenylalanine by the iron-dependent enzyme phenylalanine monooxygenase (also called hydroxylase). The reaction (shown in Figure 6.28) occurs in the liver and the kidney and requires the cosubstrate tetrahydrobiopterin. Vitamin C is thought to have a role in regenerating tetrahydrobiopterin from dihydrobiopterin [14].

Also involved in tyrosine catabolism is another hydroxylation in which ascorbate participates. Ascorbate is a preferred reductant for the copper-dependent enzyme para (p)-hydroxyphenylpyruvate hydroxylase (also called dioxygenase), the enzyme necessary for conversion of para (p)-hydroxyphenylpyruvate to homogentisate.



Finally, in tyrosine catabolism, vitamin C functions as the reductant as the compound homogentisate is converted to 4-maleylacetoacetate by the iron-dependent enzyme homogentisate dioxygenase (in right column and Figure 6.28).

Defects in this enzyme result in the disorder alkaptonuria. Alkaptonuria is characterized by an accumulation of homogentisate in the body and can lead to painful joints. Some of the homogentisate also gets excreted in the urine, and when the urine is exposed to air, the homogentisate (and thus the urine) turns black.



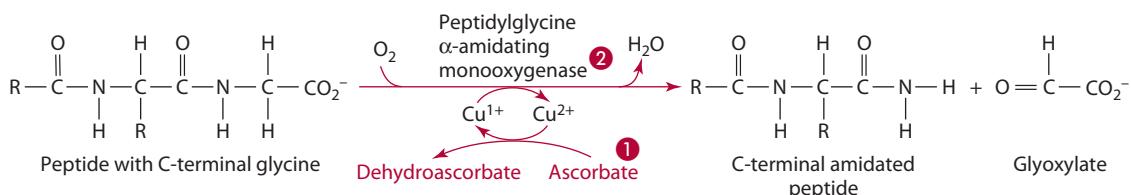
Neurotransmitter Synthesis

Ascorbate is also involved in neurotransmitter synthesis. As with the synthesis of carnitine and collagen, vitamin C maintains mineral cofactors for some of the enzymes involved in the synthesis of neurotransmitters, such as norepinephrine and serotonin, in the reduced state.

Norepinephrine Norepinephrine (a catecholamine) is generated from the hydroxylation of the side chain of dopamine in a vitamin C-dependent reaction. The reaction is catalyzed by dopamine monooxygenase, which contains eight copper atoms and is found in nervous tissue and in the adrenal medulla (Figure 6.28). The copper atoms in the enzyme are thought to act as intermediates, accepting electrons from ascorbate as they are reduced to cuprous ions (Cu^{1+}) and subsequently transferring these electrons to oxygen as they are reoxidized back to cupric ions (Cu^{2+}) [15].

Serotonin Vitamin C, tetrahydrobiopterin, and oxygen are also involved in the hydroxylation of tryptophan for the synthesis of the neurotransmitter serotonin (5-hydroxytryptamine) in the brain (Figure 6.29). Tryptophan hydroxylase, also called monooxygenase, catalyzes the first step in serotonin synthesis, whereby tryptophan is converted to 5-hydroxytryptophan in a tetrahydrobiopterin-dependent reaction. Ascorbate may help regenerate the cosubstrate tetrahydrobiopterin from dihydrobiopterin. Subsequently, 5-hydroxytryptophan is decarboxylated in a vitamin B_6 -dependent reaction to generate serotonin.

Other Neurotransmitters and Hormones Ascorbate also serves as a reductant, keeping the copper atom in peptidyl-glycine α -amidating monooxygenase in its reduced state, as shown in Figure 9.5. Although most of the substrate peptides for this enzyme have a terminal glycine residue, the enzyme is also active with peptides terminating in other amino acids. Many of the amidated peptides resulting from this reaction are active as hormones, hormone-releasing factors, or neurotransmitters. Examples include bombesin or gastrin-releasing peptide (GRP), calcitonin, cholecystokinin (CCK), thyrotropin, corticotropin-releasing factor, gastrin, growth hormone-releasing factor, oxytocin, and vasopressin [14]. The enzyme is found in neuroendocrine



- ① Vitamin C functions as a reducing agent to convert copper that has become oxidized during the reaction back to a reduced (Cu¹⁺) form.
- ② The enzyme cleaves the carboxyl-terminal residue on the peptide substrate. The residue is released as glyoxylate.

Figure 9.5 Amidation of peptides with C-terminal glycine requires vitamin C.

cells of the pituitary, adrenal, and thyroid glands and in the brain. As a reductant for the required amidating enzyme, vitamin C assumes important, although indirect, roles in many regulatory processes.

Microsomal Metabolism

A group of enzymes makes up a microsomal metabolizing system that functions mostly in liver microsomes and reticuloendothelial tissues to inactivate both endogenous and exogenous substances. Endogenous substrates include various hormones and steroids such as cholesterol. For example, cholesterol 7 α -hydroxylase, found in the microsomes of the liver, is required for the initial step in the synthesis of bile acids from cholesterol. Vitamin C plays an undefined role in this hydroxylation. Vitamin C also participates in aldosterone and cortisol synthesis.

Exogenous substrates for the microsomal metabolizing system are usually xenobiotics. *Xenos* means “stranger” in Greek, and **xenobiotics** are foreign chemicals such as drugs, carcinogens, pesticides, food additives, pollutants, or other noxious compounds. The reactions needed to metabolize these substances usually involve hydroxylations followed by conjugations or methylations to produce polar metabolites for excretion. The hydroxylation reactions are catalyzed by monooxygenases or cytochrome P₄₅₀ mixed-function oxidases and require reducing agents such as vitamin C and NAD(P)H as well as oxygen. The exact role of vitamin C, however, has not been established.

Antioxidant Activity

In addition to ascorbate's roles in collagen, carnitine, and neurotransmitter synthesis and in microsomal metabolism, vitamin C functions in a general capacity as a reducing agent or electron donor and thereby has antioxidant activity. Ascorbic acid acts as a reducing agent in aqueous solutions such as the blood and within cells. Stated slightly differently, ascorbate is an antioxidant in that it reverses oxidation. Reducing agents or antioxidants such as ascorbate may reverse oxidation by donating electrons and hydrogen ions. The reduction potential of ascorbate

is such that it readily donates electrons/hydrogen ions to regenerate other antioxidants, such as vitamin E, glutathione, and uric acid, and to reduce numerous reactive oxygen and nitrogen species [5,16–18].

As an antioxidant, ascorbate may react in blood or intracellularly with a variety of reactive oxygen and nitrogen species and give the radicals an electron in the form of a hydrogen ion. Free radicals exist independently and contain one or more unpaired electrons in an outer orbital surrounding the nucleus of the atom. Remember from chemistry that electrons are usually found in pairs in an orbital. Free radicals and other reactive oxygen species are formed during normal cellular metabolism, a process discussed in more detail in the Perspective at the end of Chapter 10. Examples of reactive oxygen species that vitamin C may reduce include:

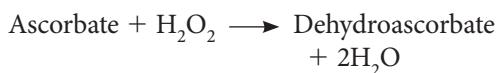
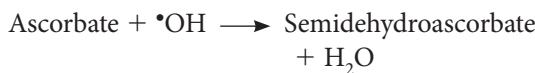
- hydroxyl radical ($\bullet\text{OH}$), a very reactive oxygen-centered radical
- hydroperoxyl radical ($\text{HO}_2\bullet$), an oxygen-centered radical
- superoxide radical ($\text{O}_2\bullet$), an oxygen-centered radical
- alkoxyl radical ($\text{RO}\bullet$), an oxygen-centered radical
- peroxy radical ($\text{RO}_2\bullet$), an oxygen-centered radical

Hydrogen peroxide, H₂O₂, a nonradical because it has no unpaired electrons in its orbital, is an example of a reactive oxygen species that, like hypochlorous acid (HOCl) and singlet oxygen ($^1\text{O}_2$), is scavenged by vitamin C. Two reactive nitrogen species, peroxynitrite radicals and nitric oxide radicals, also may be reduced by vitamin C.

Once formed, free radicals and reactive species attack nucleic acids in DNA, polyunsaturated fatty acids in phospholipids, and proteins in cells. Ascorbic acid has been shown to interact with oxidants in the aqueous phase before they initiate damage, especially to cell lipids [19–21]. Furthermore, ascorbic acid appears to be superior to other water-soluble antioxidants such as bilirubin, uric acid, and protein thiols.

The ability of plasma antioxidants to protect lipids against peroxidation has been shown to be: ascorbate = thiols > bilirubin > uric acid > vitamin E [20,22,23].

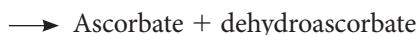
Some examples of reactions involving ascorbate as an antioxidant include these [22]:



The role of vitamin C and other antioxidants as a defense against oxidative damage to the cell is discussed in the Perspective at the end of Chapter 10.

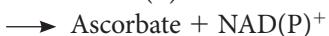
As an antioxidant, ascorbate provides electrons and becomes oxidized in the process. Regenerating ascorbic acid from semidehydroascorbate radical and from dehydroascorbate is crucial. To regenerate ascorbic acid, two semidehydroascorbate radicals may react as follows:

2 semidehydroascorbate radicals



Alternately, reductases are found in most tissues to reduce the semidehydroascorbate radical to ascorbate. Niacin and thiols such as dihydrolipoic acid, glutathione, and thioredoxin assist in vitamin C regeneration [15,22,24,25]. Niacin in its coenzyme forms, NADH and NADPH, enables vitamin C to be regenerated as follows:

2 semidehydroascorbate radicals + NAD(P)H + H⁺



Dihydrolipoic acid provides hydrogens to the dehydroascorbate form of vitamin C to recycle ascorbate, as shown here:

Dehydroascorbate + dihydrolipoic acid



Glutathione in its reduced state (GSH) functions as shown:

2 semidehydroascorbate radicals + 2GSH



Dehydroascorbate + 2GSH



Thioredoxin (Trx-[SH]₂), a dithiol, also provides reducing equivalents to dehydroascorbate, as shown here:

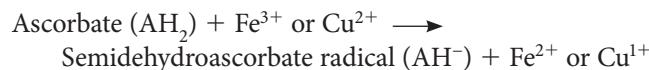
Dehydroascorbate + Trx-(SH)₂



Pro-oxidant Activity

Paradoxically, vitamin C also may act as a pro-oxidant. Vitamin C can reduce transition metals, such as cupric ions (Cu²⁺)

to cuprous (Cu¹⁺) and ferric ions (Fe³⁺) to ferrous (Fe²⁺), while itself becoming oxidized to semidehydroascorbate:



The products—Fe²⁺ and Cu¹⁺—generated from these reactions can cause cell damage by generating reactive oxygen species and free radicals. Examples of some of these reactions include:



Note that vitamin C reacts with *free* ferric or cupric ions. In the body, iron and copper are both *bound* to various proteins (i.e., not free) to minimize the likelihood of such interactions. In addition, although vitamin C appears to act as a pro-oxidant to promote lipid and cellular damage, such activity only has been shown *in vitro* and at high (nonphysiological) concentrations [11,26].

Other Functions

Many other diverse biochemical functions for vitamin C have been proposed. Experimental evidence supporting these functions varies considerably. Experimental results often conflict, and the mechanism by which ascorbate may be involved is generally unclear. Possible functions for vitamin C include roles in collagen gene expression; synthesis of bone matrix, proteoglycans, fibronectin, and elastin; regulation of cellular nucleotide (cAMP and cGMP) concentrations; and immune function, including complement synthesis [15,27,28]. Ascorbic acid also appears to be necessary for folate metabolism; specifically, vitamin C is thought to be needed to maintain folate in a reduced state either as tetrahydrofolate, the active form of the vitamin, or as dihydrofolate.

Much attention also has been directed toward vitamin C and its possible effect on diseases ranging from the common cold to cancer and heart disease, among others.

Colds The possible pharmacological effects of vitamin C on the incidence, severity, and duration of the common cold have been almost totally refuted by some investigators [29]. High doses of ascorbate appear to be only weakly prophylactic, if at all, and to be of little or no use for treating colds [29]. Vitamin C (1 g/day) had no more effect in protection against or in combating the common cold than 50 mg/day. Further, no significant differences between the groups were evident with regard to the number of colds, their severity, or their duration [29]. Other reports, however, conflict and suggest a decrease in the duration of cold episodes and the severity of symptoms [30,31]. Vitamin C is thought to moderate colds by enhancing many immune cell (such as some leukocyte) functions while also destroying histamine, which causes many of a cold's symptoms [32].

Cancer Epidemiological studies provide evidence that increased intakes of fruits and vegetables are associated with a decreased risk of some cancers [33–36]. The association between high vitamin C intake and a protective effect against cancer is generally stronger with cancers of the oral cavity, pharynx, esophagus, and stomach than with other cancers, such as of the lung, colon, pancreas, and cervix [33,36,37]. In addition, high vitamin C intakes have been inversely associated with a decreased risk (20%) of breast cancer in a meta-analysis [38]. Other studies [34–36,39,40] both support some and negate some of these associations. In clinical trials, some researchers have shown that the survival time in cancer patients could be prolonged through massive doses of vitamin C, whereas others have demonstrated no such success [33,41–43].

Possible mechanisms of ascorbate action against cancer development include roles in immunocompetence and as an antioxidant, an ability to detoxify carcinogens or block carcinogenic processes [33–35,43]. The fact that vitamin C in amounts of about 1 g, when ingested with nitrates or nitrites, can prevent formation of carcinogenic nitrosamines supports this detoxification theory and has lent credence to the vitamin's being somewhat protective against stomach and esophageal cancers [42,44,45]. Vitamin C is not unique in this regard, as other reducing agents and some food components are also effective in preventing nitrosocarcinogens [43].

Cardiovascular Disease Many (but not all) epidemiological and prospective studies report that increased fruit and vegetable intake, vitamin C intake, and plasma vitamin C concentration are associated with decreased risk of heart disease [46–57]. Low vitamin C status also is related to increased blood total cholesterol concentrations, whereas high plasma vitamin C concentrations have been associated with lower blood pressure and with higher plasma high-density lipoprotein cholesterol concentrations, both of which are protective against heart disease [58]. Yet, studies with supplements of vitamin C and other antioxidants typically have not reported beneficial effects and reviews of studies suggest minimal effects [59–64].

The mechanisms by which vitamin C may protect against heart disease in humans are not clearly identified. Animal studies suggest that vitamin C deficiency alters cholesterol metabolism, thereby affecting the generation of bile salts [15]. Vitamin C decreases monocyte adhesion to endothelial cells lining blood vessels, and adhesion represents one of the first steps in atherogenesis. Following adhesion, monocytes typically can migrate into the arterial intima (the innermost layer of blood vessels), where they become macrophages and take up oxidized cholesterol in low-density lipoproteins (LDL). Impaired vitamin C status, as well as leukocyte activation, results in increased LDL oxidation [65–67]. Macrophages take up oxidized LDL. With continued accumulation of oxidized LDL, macrophages

develop into foam cells, and over time fatty streaks develop. This sequence represents the initial steps in atherosclerosis. Vitamin C, alone and with vitamin E, may help prevent heart disease through its ability to scavenge reactive species/free radicals before they reach and initiate oxidation of LDL. Vitamin C deficiency in guinea pigs is associated with myocardial lipid peroxidative damage [17,27,65,68]. The addition of vitamin C to LDL undergoing oxidation in vitro mediated by aqueous peroxy radicals (ROO^\bullet) results in diminished use of vitamin E and diminished LDL oxidation [68]. Vitamin C concentrations 0.8 mg/dL in vitro inhibit metal-induced LDL oxidation [69]. Urinary isoprostanate concentrations (an indicator of free radical damage) were diminished in smokers receiving vitamin C supplementation [70].

Cataracts High vitamin C intakes also are thought to be beneficial in regard to diminishing cataracts and possibly age-related macular degeneration, both of which are major causes of blindness, especially in older people. Cataracts result in part from oxidative damage to proteins in the lens of the eye. The damaged proteins aggregate and precipitate, causing the lens to become cloudy. Oxygen and oxyradicals are thought to contribute to the development of cataracts. Poor antioxidant status or intake, especially of vitamins E and C and β -carotene, has been shown in many but not all studies to be associated with development of cataracts [71–79]. Although some epidemiological studies suggest a protective effect of vitamin C against cataracts and age-related macular degeneration, the effect cannot be attributed solely to vitamin C because subjects in many of the studies were consuming a multivitamin preparation [71–73,80].

INTERACTIONS WITH OTHER NUTRIENTS

Vitamin C interacts mainly with two minerals, iron and copper. The interaction between iron and vitamin C is related not only to the vitamin's effect on intestinal absorption of nonheme iron but also to the distribution of iron in the body. Specifically, ascorbate enhances the intestinal absorption of nonheme iron either by reducing iron to a ferrous (Fe^{2+}) form from a ferric (Fe^{3+}) form or by forming a soluble complex with the iron in the alkaline pH of the small intestine, thereby enhancing iron's absorption. Excessive iron in the presence of vitamin C, however, may accelerate the oxidative catabolism of vitamin C, negating the enhancing effects of vitamin C on iron absorption. Incorporation of iron into ferritin, the storage form of iron, and stabilization of ferritin by ascorbate have also been demonstrated [81]. The effect of the vitamin in the distribution and mobilization of storage iron is uncertain. Ascorbic acid supplements can cause a change in the distribution of iron in patients suffering from iron overload, but not necessarily in other people [82]. Vitamin C-initiated free radical generation

from mobilization of storage iron has been suggested but also refuted [20,73,83,84].

With respect to copper, vitamin C intakes of 1.5 g daily for about 2 months resulted in decreased serum copper and ceruloplasmin, a copper-containing protein with oxidase activity; however, despite the decrease, serum copper levels remained within normal range [85]. Dietary vitamin C intakes in excess of 600 mg daily have also been shown to decrease the oxidase activity of ceruloplasmin. Ascorbate may cause copper dissociation from ceruloplasmin or may influence the binding of copper to enzymes [42,86]. Human cells treated with vitamin C have exhibited enhanced copper uptake from ceruloplasmin [42]. Decreased intestinal absorption of copper by ascorbic acid has been observed in several animal species. A proposed mechanism of interaction for this effect suggests that vitamin C stimulated iron mobilization and the mobilized iron in turn inhibited copper absorption [86]. In addition, vitamin C may inhibit the binding of copper to metallothionein, a protein found in the intestinal cells and other body cells. It has been proposed that the delayed binding may inhibit copper transport across the intestinal cell [86].

METABOLISM AND EXCRETION

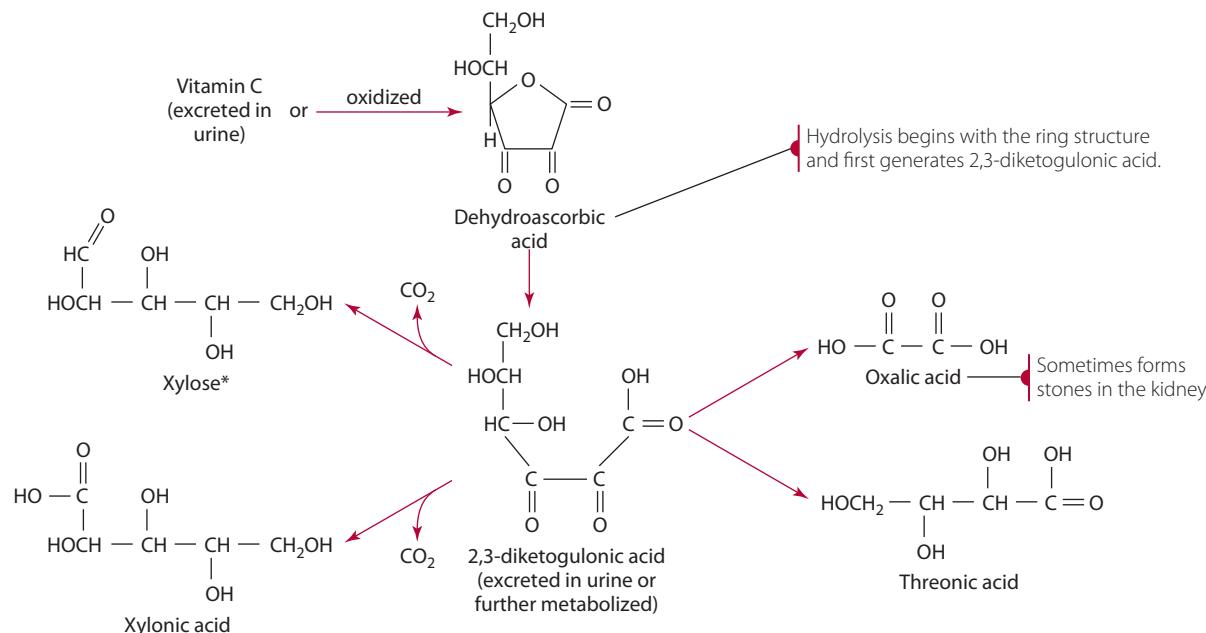
As vitamin C intakes increase, plasma vitamin C concentrations increase but reach an upper limit as renal handling of the vitamin shifts from active saturable reabsorption by SVCT1 carriers in the renal tubules to a renal threshold in which the maximum reabsorption of the vitamin is achieved. The renal reabsorption threshold occurs with

plasma vitamin C concentrations of about 1.2 mg/dL. At vitamin C intakes of about 500 mg, all vitamin C is usually excreted [61].

Vitamin C may be excreted intact or may be oxidized to dehydroascorbate. Oxidation occurs primarily in the liver but also to some extent in the kidney. Oxidation of dehydroascorbate begins with hydrolysis (opening) of the ring structure to yield 2,3-diketogulonic acid, which possesses no vitamin C activity and can be excreted in the urine or further hydrolyzed (Figure 9.6). Diketogulonate is cleaved by separate pathways either into oxalic acid and the four-carbon sugar threonic acid or into a variety of five-carbon sugars (xylose, xylonate, and lyxone). The oxalic acid is excreted in the urine, and its concentration does not appear to vary with intakes up to about 200 mg of the vitamin [6]. The four- and five-carbon sugars can be converted into cellular compounds or be oxidized and excreted as CO₂ and water. Other urinary vitamin C metabolites include 2-O-methyl ascorbate, ascorbate 2-sulfate, and 2-ketoascorbitol.

RECOMMENDED DIETARY ALLOWANCE

Current (2000) requirements for vitamin C intake are based on nearly maximizing tissue concentrations and minimizing urinary excretion of the vitamin [87]. The RDA for adult men and women is 90 mg and 75 mg, respectively, with requirements estimated at 75 mg and 60 mg, respectively [87]. Recommended intakes of 90 mg/day have been suggested by some [26]. During pregnancy and lactation, recommendations for vitamin C increase to 100 mg and



*Some of the sugars like xylose can be further metabolized before excretion.

Figure 9.6 Vitamin C and the formation of its metabolites excreted in the urine.

120 mg, respectively [87]. The 1989 RDA for the first time singled out cigarette smokers for an increased vitamin C requirement based on studies showing that smoking accelerates depletion of the body's ascorbate pool [88]. Current recommendations for smokers suggest an added 35 mg vitamin C daily [87].

DEFICIENCY

Deficient vitamin C intakes result in the deficiency condition known as **scurvy**. Scurvy is typically manifested when the total body vitamin C pools fall below about 300 mg and plasma vitamin C concentrations drop to $<0.2 \text{ mg/dL}$ [8,14]. Scurvy may be characterized by a multitude of signs and symptoms, many of which are thought to result from impaired hydroxyproline and hydroxylysine synthesis needed for collagen formation. The most notable signs and symptoms include bleeding gums, small red skin discolorations caused by ruptured small blood vessels (**petechiae**), sublingual hemorrhages, easy bruising (ecchymoses and purpurae), impaired wound and fracture healing, joint pain (arthralgia), loose and decaying teeth, and hyperkeratosis of hair follicles, especially on the arms, legs, and buttocks [89]. Scurvy is fatal if untreated. The four Hs—*hemorrhagic* signs, *hyperkeratosis* of hair follicles, *hypochondriasis* (psychological manifestation), and *hematologic* abnormalities (associated with impaired iron absorption)—are often used as a mnemonic device for remembering scurvy signs [14].

Although scurvy is rare in the United States, low plasma vitamin C levels have been observed in the elderly, especially if institutionalized. People who have poor diets, especially if coupled with alcoholism or drug abuse, are likely to be deficient, as are people with diseases such as diabetes mellitus and some cancers that increase the turnover rate of the vitamin.

TOXICITY

Daily intakes of up to 2 g vitamin C are routinely consumed without adverse effects [6,84,90]. Because vitamin C absorption is saturable and dose dependent, more vitamin C is absorbed, and thus toxicity is theoretically more likely if several large (1 g) doses of the vitamin are ingested throughout the day than if the same amount is ingested as one single dose. The most common side effect with ingestion of large amounts (2 g) of the vitamin is gastrointestinal problems characterized by abdominal pain and osmotic diarrhea. The unabsorbed vitamin C in the intestinal tract that is metabolized by bacteria within the colon is what promotes the osmotic diarrhea [3,7,42,91]. Based on this side effect, a tolerable upper intake level of 2 g vitamin C has been recommended [87].

Two other side effects reported from the use of large amounts of vitamin C are thought to affect (if at all) only

selected populations. These side effects include increased risk of kidney stones and iron toxicity for those with renal disease and disorders of iron metabolism, respectively. The possible development of kidney stones (nephrolithiasis), either oxalic acid or uric acid in content, is based on vitamin C's metabolism. Because vitamin C is metabolized in the body to oxalate and because calcium oxalate is a common constituent of kidney stones, ingestion of large doses of vitamin C has been purported as an etiologic factor in nephrolithiasis. However, although doses of up to 10 g of vitamin C have been shown to increase oxalate excretion, the amount of oxalate excreted (generally $<50 \text{ mg}$) typically has remained within a normal and safe range [91–94]. Nevertheless, some suggest that people predisposed to calcium oxalate kidney stones avoid high doses ($\geq 500 \text{ mg}$) of vitamin C [90–94]. Furthermore, because of interactions in the kidney between vitamin C and uric acid, which also is a constituent of kidney stones, people with uric acid kidney stones should avoid ingesting large doses of ascorbic acid [95]. Specifically, vitamin C competitively inhibits renal reabsorption of uric acid, thereby increasing uric acid excretion. The resulting urine acidification, along with the excessive amount of uric acid being excreted, could cause precipitation of urate crystals and urate kidney stones [95]. The actual clinical importance of uricosuria (high levels of uric acid in the urine) with regard to stone formation is unknown [90].

In addition to increasing the probability of kidney stones, chronic high doses of vitamin C are also purported to be unsafe for people with disorders involving iron metabolism, including people with **hemochromatosis**, **thalassemia**, and **sideroblastic anemia** [83,90]. However, others contend that pro-oxidant effects of vitamin C on mobilization of iron stores do not occur *in vivo* [20,41,84].

The issue of systemic conditioning to high intakes of vitamin C is currently deemed doubtful. Although scurvy-like symptoms were reported in a few people on abrupt withdrawal of large intakes of vitamin C, the reports are anecdotal. Further substantiation of conditioned (also called rebound) scurvy is needed before recommendations can be made [84,87].

Excessive ascorbate excretion can interfere with some clinical laboratory tests. Vitamin C in the urine, for example, may act as a reductive agent and thus interfere with diagnostic tests using redox chemistry. For example, tests for glucose in the urine can be rendered invalid, false-negative tests for fecal occult blood may be generated, and occult blood in the urine may not be detected [42].

ASSESSMENT OF NUTRITION

Plasma and serum vitamin C concentrations respond to changes in dietary vitamin C intakes and thus are used to

assess recent vitamin C intake. White blood cell (WBC) content of the vitamin better reflects body stores, but this measurement is technically more difficult to perform. Plasma concentrations of vitamin C below 0.2 mg/dL are considered to be deficient. Concentrations associated with tissue saturation are about 1.0 mg/dL, and those typically found with recommended intakes range from about 0.6 to 0.8 mg/dL [6]. Leukocyte vitamin C concentrations of 10 µg/10⁸ WBC or less are considered deficient [96].

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Thiamin (Vitamin B₁)

Thiamin (vitamin B₁), the structural formula of which is shown in Figure 9.7, consists of a pyrimidine ring and a thiazole moiety (meaning one of two parts) linked by a methylene (CH₂) bridge.

The need for thiamin was first realized in the late 1800s by a Dutchman, C. Eijkman, when it was discovered that fowl fed a diet of cooked, polished (devoid of the outer layers) rice developed neurologic problems (now called beriberi). The substance initially called thiamine that corrected the problems was first isolated from rice bran in 1912 by Casimir Funk. The vitamin's structure (discovered by R. Williams from the United States) was not determined until about the mid 1930s.

SOURCES

Thiamin is widely distributed in foods, including meat (especially pork), legumes, and whole, fortified, or enriched grain products, cereals, and breads. Yeast, wheat germ, and soy milk also contain significant amounts of the vitamin. In supplements, thiamin is found mainly as thiamin hydrochloride or as thiamin mononitrate salt.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

In plants, thiamin exists in a free (nonphosphorylated) form. However, in animal products, 95% of thiamin occurs in a phosphorylated form, primarily thiamin diphosphate (TDP), also called thiamin pyrophosphate (TPP). Intestinal phosphatases hydrolyze the phosphates from the thiamin diphosphate prior to absorption.

Absorption of thiamin from foods is thought to be high. Occasionally, however, antithiamin factors may be present in the diet. For example, thiaminases present in raw fish catalyze the cleavage of thiamin, destroying the vitamin. These thiaminases are thermostable, so cooking fish renders the enzymes inactive. Other antithiamin factors include polyhydroxyphenols such as tannic and

cafeic acids. Polyhydroxyphenols, which are thermostable, are found in coffee, tea, betel nuts, and certain fruits and vegetables such as blueberries, black currants, Brussels sprouts, and red cabbage. These polyhydroxyphenols inactivate thiamin by an oxyreductive process; the destructive process can be facilitated by the presence of divalent minerals such as calcium and magnesium. Thiamin destruction may be prevented, however, by the presence of reducing compounds such as vitamin C and citric acid.

Absorption of thiamin occurs primarily in the jejunum, with lesser amounts absorbed in the duodenum and ileum. Free thiamin, not phosphorylated thiamin, is absorbed into the intestinal mucosal cells. Yet, within the mucosal cells, thiamin may be phosphorylated (i.e., converted into a phosphate ester). Absorption of thiamin can be both active and passive, depending on the amount of the vitamin presented in the intestine for absorption. When intakes of thiamin are high, absorption is predominantly by passive diffusion [1]. At low physiological concentrations, thiamin absorption is active and is sodium-dependent [1]. Other studies, however, have demonstrated sodium independent carrier-mediated transport [2–4]. Two thiamin transporters from the SLC19 gene family have been characterized; the protein carriers are called ThTr1 and ThTr2. Both of these carriers are found in a variety of tissues including the intestine and kidneys and are thought to exchange thiamin for H⁺ ions as part of an antiport carrier system [5–7]. Defects in the gene SLC19A2, which codes for ThTr1, have been shown to cause thiamin deficiency [6].

Thiamin transport across the basolateral membrane occurs by a thiamin/H⁺ antiport system [4]. Ethanol ingestion, however, interferes with active transport of thiamin from the mucosal cell across the basolateral membrane, but not the brush border membrane.

Thiamin in the blood is typically either in its free form, bound to albumin, or found as thiamin monophosphate (TMP). Thiamin appearing on the serosal side of the enterocyte is not, however, initially bound to phosphates. Most (~90%) of the thiamin in the blood is present within the blood cells. Transport of thiamin into red blood cells is thought to occur by facilitated diffusion, whereas transport into other tissues requires energy. Only free thiamin or TMP is thought to be able to cross cell membranes. In red blood cells, most thiamin exists as TDP, with smaller amounts of free thiamin and TMP.

The human body contains approximately 30 mg thiamin, with relatively high but still small concentrations found (stored) in the liver, skeletal muscles, heart, kidney, and brain. In fact, skeletal muscles are thought to contain about half of the body's thiamin.

Following absorption, most free thiamin is taken up by the liver and phosphorylated, such that thiamin is

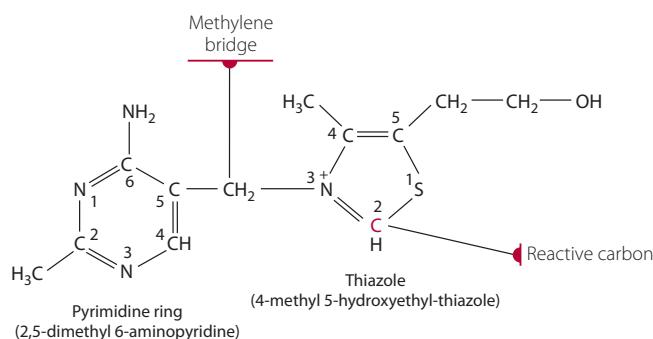
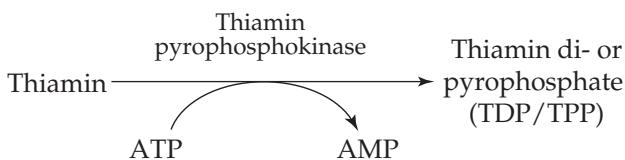
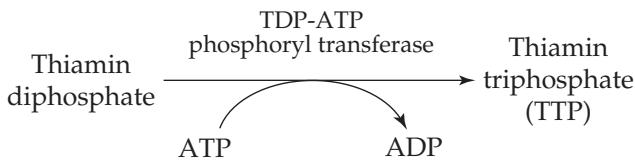


Figure 9.7 Structure of thiamin.

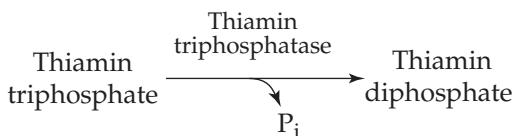
converted to its coenzyme phosphorylated form, thiamin diphosphate (TDP). Conversion of thiamin to TDP requires adenosine triphosphate (ATP) and thiamin pyrophosphokinase, an enzyme found in the liver, brain, and other tissues. About 80% of the total thiamin in the body exists as TDP.



Another form of thiamin, thiamin triphosphate (TPP), represents about 10% of total body thiamin. TPP is synthesized by action of a TDP-ATP phosphoryl transferase that phosphorylates TDP.



The terminal phosphate on the TPP may be hydrolyzed by thiamin triphosphatase to yield TDP.



TDP can be converted to TMP by thiamin diphosphatase. TMP can be then converted to free thiamin by thiamin monophosphatase.

TPP, as well as TDP and TMP, can be found in small amounts in several tissues, including the brain, heart, liver, muscles, and kidney. TMP is thought to be derived from the catabolism of the terminal phosphate on TDP and is believed to be inactive. Enzymes responsible for thiamin phosphorylation and dephosphorylation are found in a variety of organs and tissues, including the brain.

FUNCTIONS AND MECHANISMS OF ACTION

Thiamin plays essential coenzyme and noncoenzyme roles in the body, including these:

- energy transformation (a coenzyme role)
- synthesis of pentoses and nicotinamide adenine dinucleotide phosphate (NADPH) (also as a coenzyme role)
- membrane and nerve conduction (in a noncoenzyme capacity)

Each of these three roles is discussed in this section.

Coenzyme Roles

As TDP, thiamin functions in energy transformation as a coenzyme of the pyruvate dehydrogenase complex, the α -ketoglutarate dehydrogenase complex, and the branched-chain α -keto acid dehydrogenase complex. In addition, TDP serves as a coenzyme for transketolase needed for the synthesis of NADPH and pentoses. The reactions are shown as an overview in Figure 9.8 and discussed in detail in the next section.

Energy Transformation Thiamin as TDP functions as a coenzyme necessary for the oxidative decarboxylation of pyruvate, α -ketoglutarate, and the three branched-chain amino acids isoleucine, leucine, and valine. These reactions are instrumental in generating energy (ATP). Inhibition of the decarboxylation reactions of especially pyruvate and α -ketoglutarate prevents synthesis of ATP and of the acetyl CoA needed for the synthesis of, for example, fatty acids, cholesterol, and other important compounds. The inhibition also results in the accumulation of pyruvate, lactate, and α -ketoglutarate in the blood.

The steps that occur in the oxidative decarboxylation of pyruvate to form acetyl CoA, shown in Figure 9.9, require a multienzyme complex known as the pyruvate dehydrogenase complex, which is bound to the mitochondrial membrane. Three enzymes make up the pyruvate dehydrogenase complex: a TDP-dependent pyruvate decarboxylase; a lipoic acid-dependent dihydrolipoyl transacetylase; and an FAD-dependent dihydrolipoyl dehydrogenase. The roles of four vitamins—thiamin (TDP), riboflavin (FAD), niacin (NAD^+), and pantothenic acid (CoA-SH)—in this decarboxylation process are described briefly and shown in Figure 9.9. ATP and Mg^{2+} also are required.

In the first reaction (Figure 9.10), the carbon atom between the nitrogen and sulfur atoms in the thiazole ring of TDP ionizes (deproteinizes) to form (at carbon 2 of the thiazole ring) a carbanion. The carbanion is stabilized by the positively charged nitrogen in the thiazole ring [8]. The carbanion can combine with the 2-carbonyl group of pyruvate (Figure 9.10), α -ketoglutarate, and other α -keto acids to form a covalent bond and produce an adduct or additional compound [8]. After forming an adduct between TDP and pyruvate, pyruvate decarboxylase (first enzyme of the pyruvate dehydrogenase complex) catalyzes the removal of the COO group from pyruvate forming hydroxyethyl-TDP (Figure 9.9). The hydroxyethyl group is then transferred to oxidized lipoamide (which is bound to the second enzyme dihydrolipoyl transacetylase) forming acetyl lipoamide. The acetyl lipoamide then reacts with coenzyme A to form acetyl CoA and reduced lipoamide. Lipoamide is oxidized by the third enzyme, dihydrolipoyl dehydrogenase, which requires FAD. NAD^+ oxidizes FADH_2 . Thus, the overall reaction is: Pyruvate + $\text{NAD}^+ + \text{CoA} \longrightarrow \text{Acetyl CoA} + \text{NADH} + \text{H}^+ + \text{CO}_2$.

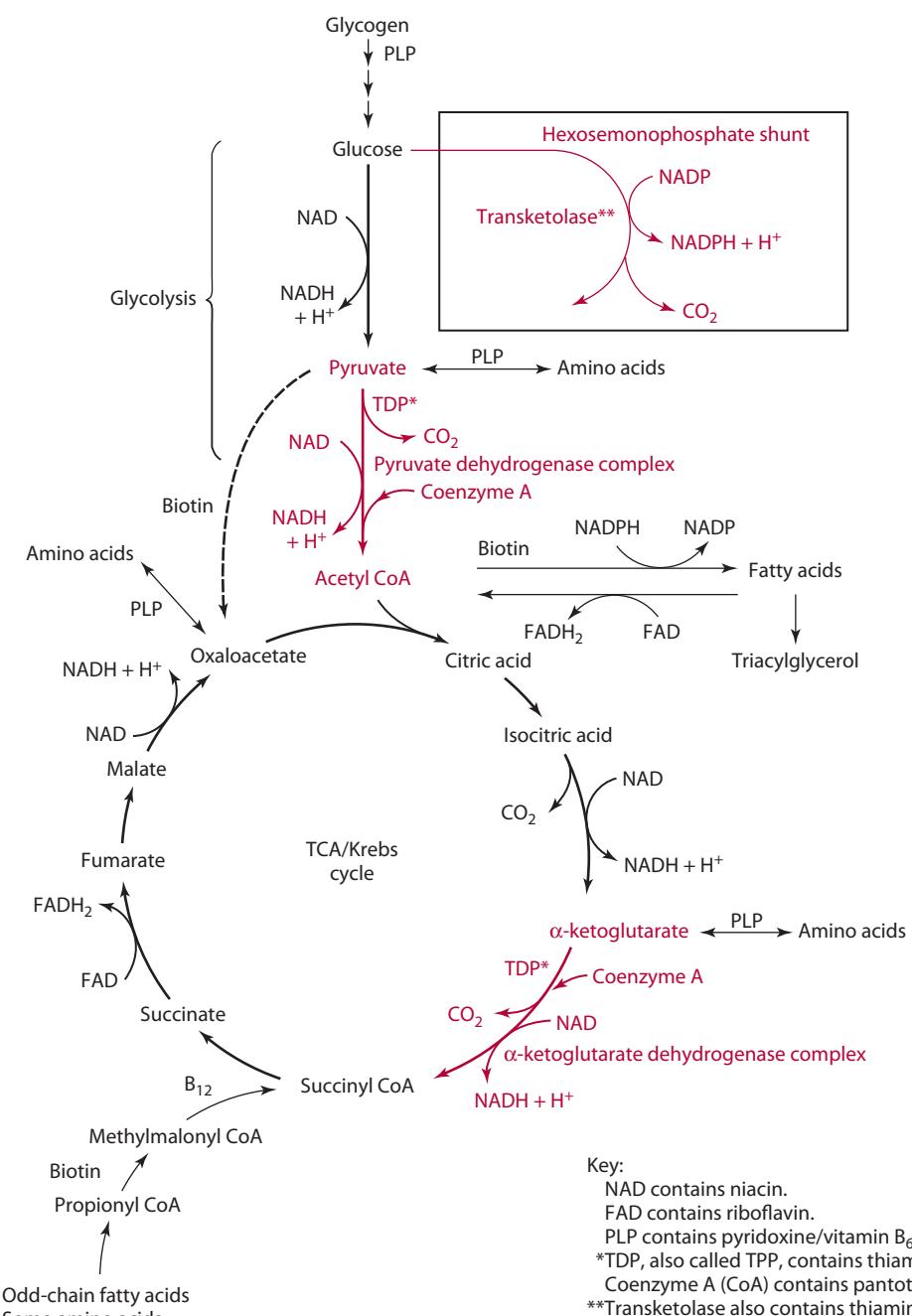
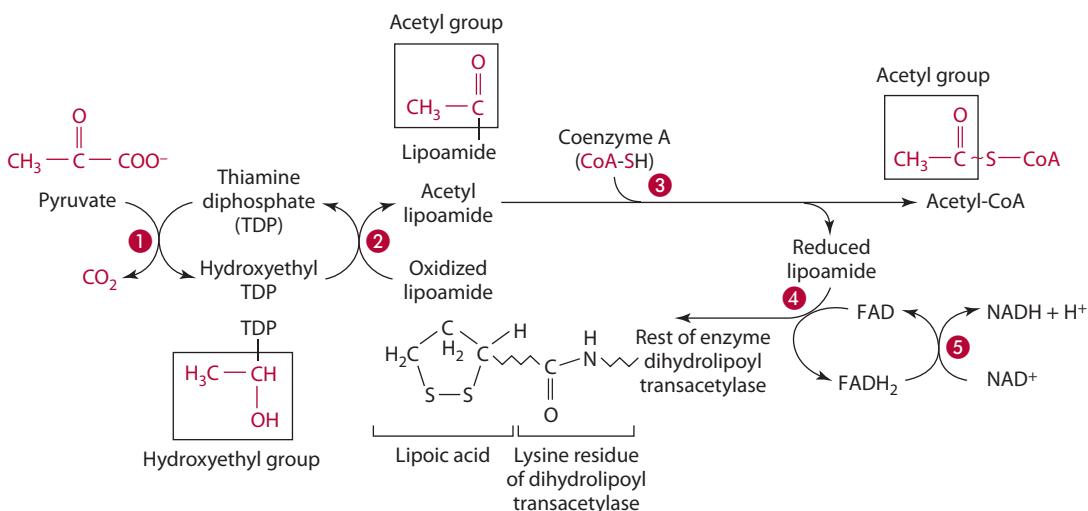


Figure 9.8 Various vitamin cofactors and their action sites in energy metabolism. The role of thiamin as TDP is shown by an asterisk.

The decarboxylation of α-ketoglutarate by the α-ketoglutarate dehydrogenase and the decarboxylation of the branched-chain keto acids by the branched-chain ketoacid dehydrogenase complex are similar to that of pyruvate. The α-ketoglutarate dehydrogenase complex decarboxylates α-ketoglutarate and forms succinyl CoA. Decarboxylation of the branched-chain α-keto acids, which arise from the transamination of valine, isoleucine, and leucine, is an oxidative process that also requires thiamin as TDP/TPP (see Figure 6.37). Failure to oxidize the α-keto acids α-ketoisocaproic, α-keto β-methylvaleric, and α-ketoisovaleric acids from leucine,

isoleucine, and valine, respectively, causes both the branched-chain amino acids and their α-keto acids to accumulate in blood and other body fluids. Such findings are characteristic of maple syrup urine disease (MSUD), an inborn error of metabolism that results from a genetic absence of or insufficient activity of the branched-chain α-keto acid dehydrogenase enzyme complex. People with MSUD must limit their intake of protein-containing foods to curtail intake of leucine, isoleucine, and valine. Medical foods devoid of these three amino acids provide most of the nutrient intake for those with MSUD.

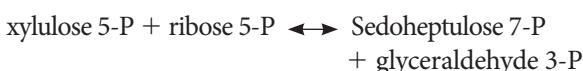


- ① CO_2 is removed from pyruvate and the rest of the compound (hydroxyethyl) attaches to TDP to form hydroxyethyl TDP.
- ② The hydroxyethyl group is transferred to oxidized lipoamide which consists of lipoic acid attached by an amide link ($\text{CO}-\text{NH}$) to a lysine residue of the enzyme dihydrolipoyl transacetylase. With the transfer of the hydroxyethyl group acetyl lipoamide is generated.
- ③ Acetyl lipoamide reacts with coenzyme A (CoA-SH) to form acetyl CoA and reduced lipoamide.
- ④ Reduced lipoamide is oxidized by the flavo (FAD) dependent enzyme dihydrolipoyl dehydrogenase.
- ⑤ The reduced flavo (FADH_2) protein is oxidized by NAD^+ which then transfers reducing equivalents to the respiratory chain.

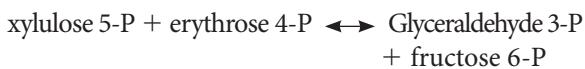
Figure 9.9 The oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex.

Synthesis of Pentoses and NADPH Thiamin as TDP also functions as a loosely bound prosthetic group of transketolase, a key cytoplasmic enzyme in the hexose monophosphate shunt. The hexose monophosphate shunt is the pathway in which sugars of varying chain lengths are interconverted (Figure 3.12). The shunt is essential for the generation of pentoses for nucleic acid synthesis and of NADPH, which is needed, for example, for fatty acid synthesis. TDP forms a carbanion that transfers an activated aldehyde from a donor ketose substrate to an acceptor. The acceptor in the hexose monophosphate shunt is xylulose. Transketolase hydrolyzes the carbon-to-carbon bond in xylose 5-P, sedoheptulose 7-P, and fructose 6-P (i.e., ketoses) and transfers the two-carbon fragment (carbons 1 and 2 of the ketoses) to an aldose receptor [8]. The transketolase catalyzed reactions are Mg^{2+} dependent and can be written as follows:

Transketolase



Transketolase



Noncoenzyme Roles: Membrane and Nerve Conduction

In addition to its coenzyme roles, thiamin, as TTP, is thought to function in a manner other than as a coenzyme. In nerve

membranes, TTP is thought to activate ion (specifically chloride) transport [3–5]. Thiamin also may be involved in nerve impulse transmission by regulation of sodium channels and acetylcholine receptors [9–11].

METABOLISM AND EXCRETION

Thiamin in excess of tissue needs and storage capacity is excreted intact, as well as catabolized for urinary excretion. Degradation of thiamin begins when the molecule is cleaved into its pyrimidine and thiazole moieties. The two rings are then further catabolized, generating 20 or more metabolites including, for example, 4-methyl thiazole 5-acetic acid and 2-methyl 4-amino 5-pyrimidine carboxylic acid. TDP and TMP are also excreted intact.

RECOMMENDED DIETARY ALLOWANCE

As with the 1989 RDA for thiamin, the basis for the 1998 RDA relies on the results of numerous metabolic studies examining urinary excretion of thiamin, changes in erythrocyte transketolase activity, and thiamin intake data. The 1998 RDA for thiamin for adult men is 1.2 mg/day and for adult women is 1.1 mg daily; the requirements for adult men and women are 1.0 mg/day and 0.9 mg/day, respectively [12]. Differences in thiamin needs between men and women are based on differences in body size and energy needs. Thiamin intakes with pregnancy and lactation increase to 1.4 mg/day and 1.5 mg/day,

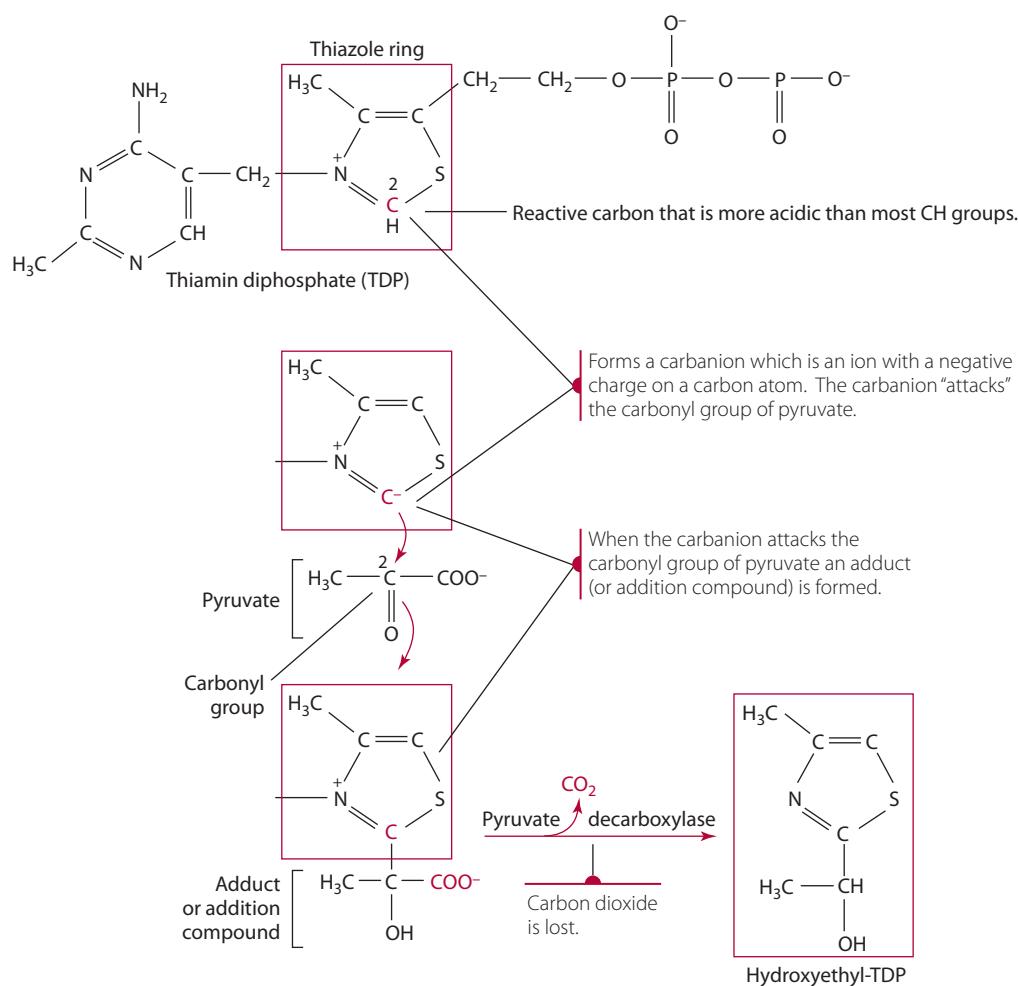


Figure 9.10 The first steps in the decarboxylation of pyruvate by thiamin diphosphate. See page 324 for a description of the reactions.

respectively [12]. The inside covers of the book provide additional RDAs for thiamin for other age groups.

DEFICIENCY: BERIBERI

Despite the known functional roles of thiamin at the cellular level, it has as yet been impossible to explain all the pathophysiological manifestations in animals or humans that are associated with thiamin deficiency, or **beriberi** (*beri* means “weakness”). One of the first symptoms of thiamin deficiency is a loss of appetite (anorexia) and thus weight loss. As the deficiency worsens, cardiovascular system involvement (such as hypertrophy and altered heart rate) and neurological symptoms (such as apathy, confusion, decreased short-term memory, and irritability) appear.

Three types of beriberi have been identified. Dry beriberi, found predominantly in older adults, is thought to result from a chronic low thiamin intake, especially if coupled with a high carbohydrate intake. Dry beriberi is characterized by muscle weakness and wasting, especially in the lower extremities, and peripheral neuropathy. The

neuropathy consists of symmetrical sensory and motor nerve conduction problems mostly affecting the distal parts of the limbs (i.e., the ankles, feet, wrists, and hands). Wet beriberi results in more extensive cardiovascular system involvement than dry beriberi; cardiomegaly (enlarged heart), rapid heart beat (tachycardia), right-side heart failure with secondary respiratory involvement, and peripheral edema are common symptoms along with peripheral neuropathy. Acute beriberi, seen mostly in infants, has been documented in countries including Japan. Acute beriberi is associated with anorexia, vomiting, lactic acidosis (the lack of thiamin, which is needed to convert pyruvate to acetyl CoA, causes pyruvate to be converted to lactic acid; the lactic acid then accumulates, causing acidosis), altered heart rate, and cardiomegaly. Use of parenteral nutrition devoid of thiamin can cause acute thiamin deficiency within a few weeks.

In the United States and in Western countries, thiamin deficiency is often associated with alcoholism. Wernicke's encephalopathy or Wernicke-Korsakoff syndrome, a neuropsychological complication, is also commonly found in those

with alcoholism and AIDS, and in those receiving parenteral (intravenous) nutrition that is high in dextrose and low or absent in thiamin [13]. People with alcohol dependency are particularly prone to thiamin deficiency because of:

- decreased intake of the vitamin from decreased food consumption
- increased requirement for the vitamin because of liver damage (decreased liver function impairs TDP formation and, consequently, vitamin use)
- decreased thiamin absorption [14]

Wernicke's encephalopathy is characterized by **ophthalmoplegia** (paralysis of the ocular muscles), **nystagmus** (constant, involuntary eyeball movement), **ataxia** (impaired muscle coordination), loss of recent memory, and confusion [14]. Thiamin deficiency also is fairly prevalent in people with congestive heart failure [15]; the higher prevalence is thought to be attributable to low intakes and increased urinary thiamin losses secondary to diuretic use. Treatment consists of therapeutic oral doses (~100 mg or more) of thiamin or intravenous doses of about 50 mg or more of thiamin. Typically, some aspects of confusion and ophthalmoplegia begin to improve with the massive thiamin doses [14].

Elderly populations are also at risk for thiamin deficiency. People with diseases that impair absorption of the vitamin (e.g., some gastrointestinal tract cancers, biliary disease, inflammatory bowel diseases) are also at greater risk of developing deficiency. Excess glucose infusion intravenously and ingestion of diets that are made up primarily of refined, unenriched grain products necessitate increased thiamin intake.

TOXICITY

There appears to be little danger of thiamin toxicity associated with oral intake of large amounts (500 mg daily for 1 month) of thiamin [16,17]. Excessive thiamin (100 times recommendations) administered intravenously or intramuscularly, however, has been associated with headache, convulsions, cardiac arrhythmia, and anaphylactic shock, among other signs [12]. No tolerable upper intake level has been established [12].

Pharmacological levels of thiamin are used in treating certain inborn errors of metabolism. For example, one variant form of maple syrup urine disease has been shown to respond to oral thiamin supplements (up to 500 mg daily). Other metabolic diseases that may respond to large doses of the vitamin are thiamin-responsive megaloblastic anemia and thiamin-responsive lactic acidosis. Although the role of thiamin in correcting anemia is not clear, in lactic acidosis large doses of thiamin increase hepatic pyruvate dehydrogenase activity, thereby decreasing pyruvate conversion to lactic acid as more pyruvate is decarboxylated to acetyl CoA for entry into the TCA cycle.

ASSESSMENT OF NUTRITION

Adequacy of thiamin nutriture can be assessed by measuring erythrocyte transketolase activity in hemolyzed whole blood or by measuring thiamin in the blood or urine [18,19]. Urinary thiamin excretion decreases with decreased thiamin status; excretion also is correlated with intake [20]. Urinary thiamin excretion <40 µg or <27 µg/g creatinine suggests thiamin deficiency. Transketolase is the thiamin-dependent enzyme of the hexose monophosphate shunt. In cases of thiamin deficiency, the enzyme increases activity with the addition of thiamin to the incubation medium. An increase in transketolase activity of >25% indicates thiamin deficiency; an increase in activity of 15% to 25% suggests marginal status; and an increase of <15% suggests adequate status. Transketolase concentrations of <120 nmol/L also have been used to indicate deficiency; concentrations of 120–150 nmol/L suggest marginal thiamin status.

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Riboflavin (Vitamin B₂)

Riboflavin consists of flavin (isoalloxazine ring), to which is attached a ribitol (sugar alcohol) side chain.

The structures of riboflavin and its two coenzyme derivatives, FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide), are given in Figure 9.11.

Riboflavin was isolated and then later synthesized. Kuhn and coworkers are credited with determining its structure along with Szent-Györgyi and Wagner-Jaunery in 1933.

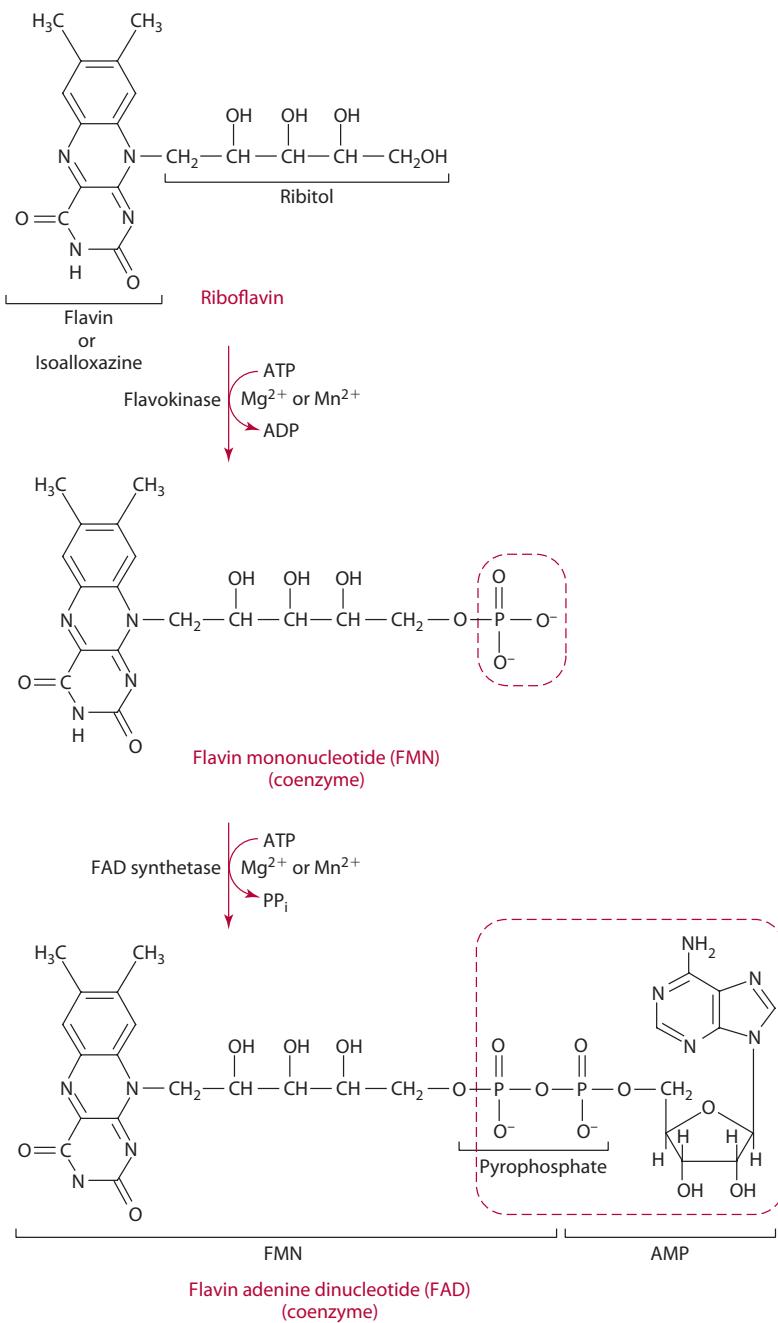


Figure 9.11 Structures of riboflavin and its coenzyme forms.

The name riboflavin signifies the presence of a ribose like side chain (ribo) and its yellow color (*flavus* means “yellow” in Latin).

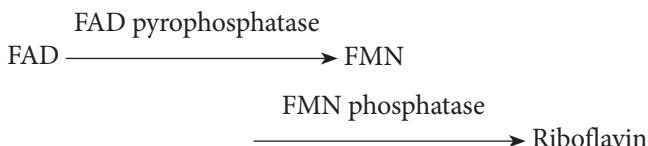
SOURCES

Riboflavin is found in a wide variety of foods, but especially those of animal origin. Milk and milk products such as cheeses are thought to contribute most dietary riboflavin. Eggs, meat, and legumes also provide riboflavin in significant quantities. Green vegetables like spinach provide fairly good riboflavin content. Fruits and cereal grains are minor contributors of dietary riboflavin.

The form of riboflavin in food varies. Free or protein-bound riboflavin is found in milk, eggs, and enriched breads and cereals. In most other foods the vitamin occurs as one or the other of its coenzyme derivatives, FMN or FAD, although phosphorus-bound riboflavin is also found in some foods.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Riboflavin attached noncovalently to proteins may be freed by the action of hydrochloric acid secreted within the stomach and by gastric and intestinal enzymatic hydrolysis of the protein. Riboflavin in foods as FAD, FMN, and riboflavin phosphate must also be freed prior to absorption. Within the intestinal lumen, FAD pyrophosphatase converts FAD to FMN; FMN in turn is converted to free riboflavin by FMN phosphatase.



Other intestinal phosphatases, such as nucleotide diphosphatase and alkaline phosphatase, are thought to hydrolyze riboflavin from riboflavin phosphate.

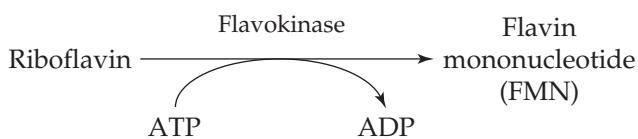
Not all bound riboflavin is hydrolyzed and available for absorption. A small amount (~7%) of FAD is covalently bound to either of two amino acids, histidine or cysteine. For example, following consumption of foods containing succinate dehydrogenase or monoamine oxidase, these proteins are degraded; however, the riboflavin remains bound, typically to histidine or cysteine residues, and cannot function in the body [1]. Should absorption of the histidine- and cysteine-bound riboflavin occur, the complex is excreted unchanged in the urine.

Generally, animal sources of riboflavin are thought to be better absorbed than plant sources. Divalent metals such as copper, zinc, iron, and manganese have been shown to chelate (bind to) riboflavin and FMN and to inhibit

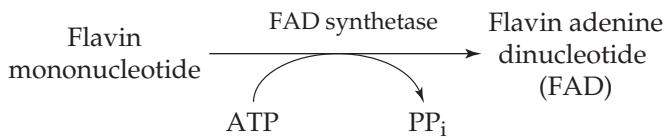
riboflavin absorption. Ingesting alcohol also impairs riboflavin digestion and absorption [2].

Free riboflavin is absorbed by a saturable, energy-dependent carrier mechanism primarily in the proximal small intestine [3]. It also has been reported that absorption occurs by sodium-independent carriers [4]. When large amounts of the vitamin are ingested, riboflavin may be absorbed by diffusion. Absorption rate is proportional to dose. About 95% of riboflavin intake from foods is absorbed, up to a maximum of about 25 mg [1,5]. Peak concentrations of the vitamin in the plasma correlate with intakes of 15 to 20 mg [6].

On absorption into the intestinal cells, riboflavin is phosphorylated to form FMN, a reaction catalyzed by flavokinase and requiring ATP, as shown here and in Figure 9.11.



At the serosal surface, most of the FMN is probably dephosphorylated by a nonspecific alkaline phosphatase to riboflavin, which enters portal blood for transport to the liver. The vitamin is carried to the liver, where it is converted again to FMN by flavokinase and to its other coenzyme derivative, FAD, by FAD synthetase (below and Figure 9.11). FAD is the predominant flavoenzyme in tissues.



Most flavins in systemic plasma are found as riboflavin rather than as one of its coenzyme forms, although all three may be present. Riboflavin, FMN, and FAD are transported in the plasma by a variety of proteins, including albumin, fibrinogen, and globulins (principally immunoglobulins) [7]. Albumin appears to be the primary transport protein. Immunoglobulins have been shown to use riboflavin to activate the antibody-catalyzed water oxidation pathway in which singlet oxygen, ${}^1\text{O}_2^\bullet$ (derived from, for example, activated white blood cells) and water react to form hydrogen peroxide [8]. Hydrogen peroxide assists in the destruction of foreign antigens, although it is also destructive to human cells.

Regardless of the form in which the vitamin reaches the tissues, free riboflavin is the form that traverses most cell membranes by a carrier-mediated process (i.e., requiring a riboflavin-binding protein). Some riboflavin carriers in some tissues such as the liver appear to be regulated by

calcium/calmodulin [4]. Diffusion, however, may also contribute when riboflavin concentrations are high. Riboflavin is found in small quantities in a variety of tissues. The greatest concentrations of riboflavin are found in the liver, kidney, and heart.

Although it is free riboflavin that is transported into and out of cells, within cells riboflavin is typically converted to its coenzyme forms by flavokinase and FAD synthetase, both of which are widely distributed in tissues, especially the liver, small intestine, kidneys, and heart [9]. Synthesis of FMN and FAD appears to be under hormonal regulation. Hormones shown to be particularly important in this regulation are ACTH, aldosterone, and the thyroid hormones, all of which accelerate the conversion of riboflavin into its coenzyme forms, apparently by increasing the activity of flavokinase [10,11]. Synthesis of the coenzymes is also regulated by product inhibition in the case of FAD synthesis [12]. Following synthesis of the flavin coenzymes, the coenzyme forms of the vitamin become bound to apoenzymes. FMN and FAD function as prosthetic groups for enzymes involved in oxidation reduction reactions. These enzymes are called flavoproteins.

FUNCTIONS AND MECHANISMS OF ACTION

FMN and FAD function as coenzymes for a wide variety of oxidative enzyme systems and remain bound to the enzymes during the oxidation-reduction reactions. Flavins can act as oxidizing agents because of their ability to accept a pair of hydrogen atoms. The isoalloxazine ring is reduced by two successive one-electron transfers with the intermediate formation of a semiquinone free radical, as shown in Figure 9.12. Reduction of the isoalloxazine ring yields the reduced forms of the flavoprotein, which can be found in FMNH_2 and FADH_2 .

Flavoproteins

Flavoproteins exhibit a wide range of redox potentials and therefore can play a wide variety of roles in intermediary metabolism. Some of these roles are discussed here.

- The role of flavoproteins in the *electron transport chain* is provided in Figures 3.26 and 3.29.

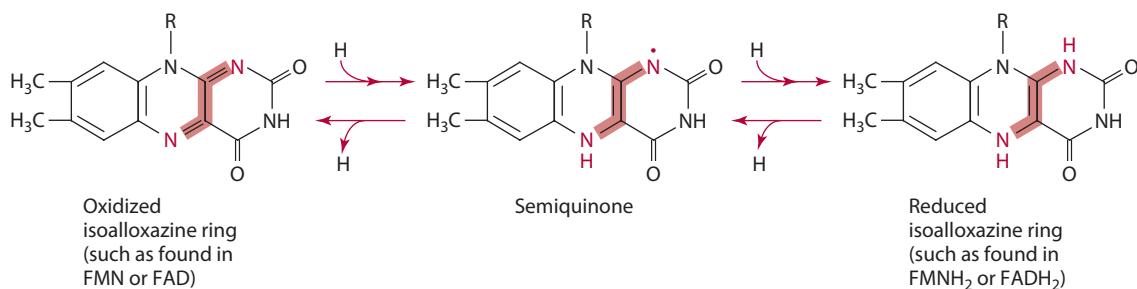
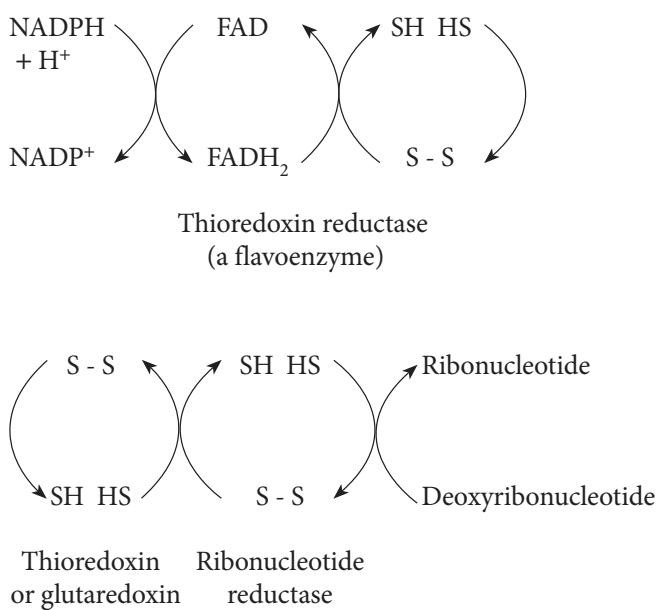


Figure 9.12 Oxidation and reduction of isoalloxazine ring.

- In the *oxidative decarboxylation of pyruvate* (Figure 9.9) and α -ketoglutarate, FAD serves as an intermediate electron carrier, with NADH being the final reduced product.
- Succinate dehydrogenase* is an FAD flavoprotein that removes electrons from succinate to form fumarate and that forms FADH_2 from FAD (Figure 3.27). The electrons are then passed into the electron transport chain by coenzyme Q (Figure 3.26).
- In fatty acid oxidation, fatty *acyl CoA* dehydrogenase requires FAD (Figure 5.23).
- Sphinganine oxidase*, in sphingosine synthesis, requires FAD.
- As a coenzyme for an oxidase such as *xanthine oxidase*, FAD transfers electrons directly to oxygen with the formation of hydrogen peroxide. Xanthine oxidase, which contains both iron and molybdenum, is necessary for purine catabolism in the liver. The enzyme converts hypoxanthine to xanthine and then xanthine to uric acid (see the section on molybdenum, Chapter 12).
- Similarly, *aldehyde oxidase* using FAD converts aldehydes, such as pyridoxal (vitamin B_6) to pyridoxic acid, an excretory product, and retinal (vitamin A) to retinoic acid, while also passing electrons to oxygen and generating hydrogen peroxide.
- Also in vitamin B_6 metabolism (seen later in Figure 9.36), *pyridoxine phosphate oxidase*—which converts pyridoxamine phosphate (PMP) and pyridoxine phosphate (PNP) to pyridoxal phosphate (PLP), the primary coenzyme form of vitamin B_6 —is dependent on FMN.
- Synthesis of an *active form of folate*, 5-methyl THF, requires FADH_2 (shown later in Figure 9.28).
- A step in the *synthesis of niacin from tryptophan* that is catalyzed by kynureninase monooxygenase requires FAD (see Figure 9.15, on page 335).
- In *choline catabolism*, several enzymes such as choline dehydrogenase, dimethylglycine dehydrogenase, and sarcosine (also called monomethylglycine) dehydrogenase require FAD.
- Some neurotransmitters (such as dopamine) and other amines (tyramine and histamine) require FAD-dependent *monoamine oxidase* for metabolism.

- Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) is also dependent on FAD-dependent *glutathione reductase*. This reaction forms the basis of one assay used to assess riboflavin status (see the section “Assessment of Nutriture”).
- Ero1 and sulphydryl oxidase are FAD dependent and help to form disulfide bonds and, thus, the structure or folding of secretory proteins. Impaired oxidative folding and subsequently impaired secretion of proteins have been shown with riboflavin deficiency [13].
- Thioredoxin reductase* is a flavo (FAD) enzyme that contains selenocysteine at its active site and transfers reducing equivalents from NADPH through its bound FAD to reduce disulfide bonds within the oxidized form of thioredoxin. The enzyme works as part of a complex set of reactions with ribonucleotide reductase in the synthesis of deoxyribonucleotides from ribonucleotides as shown here:



Ribonucleotide reductase (which contains thiol groups) catalyzes the conversion of ribonucleotides to deoxyribonucleotides (such as dADP, dGDP, dCDP, and dUDP; see the section in Chapter 6 on purines and pyrimidines), which are needed for DNA synthesis. In the reaction, the sulphydryl groups in ribonucleotide reductase become oxidized forming a disulfide bond. Thioredoxin (or glutaredoxin—a small protein like thioredoxin) provides electrons (H), but upon donation becomes oxidized itself (containing a disulfide bond). The flavoenzyme thioredoxin reductase (or glutaredoxin reductase), which also contains sulphydryl groups, reduces the thioredoxin (or glutaredoxin). NADPH then reduces the thioredoxin reductase (or glutaredoxin reductase) to eliminate the disulfide bond and regenerate the sulphydryl groups.

METABOLISM AND EXCRETION

Riboflavin and its metabolites are excreted primarily in the urine, with only small amounts lost in the feces. Fecal riboflavin metabolites may also arise from the catabolism of riboflavin by intestinal flora [1]. It is believed that some of these metabolites formed in the intestinal tract by bacteria also can be absorbed but then are excreted in the urine [1].

The riboflavin that is not bound to proteins in the plasma is filtered by the glomerulus and excreted. Most riboflavin (~60%–70%) is excreted intact in the urine in amounts >120 µg/day or 80 µg/g creatinine with adequate riboflavin intake. Metabolites arise from tissue degradation of covalently bound flavins as well as from degradation of the vitamin. Metabolites present in the greatest concentrations in the urine include 7α- and 8α-hydroxymethyl riboflavin, 8α-sulfonyl riboflavin, 10-hydroxyethyl flavin, and riboflavin peptide ester. Riboflavin bound to cysteine and histidine is also found in the urine if absorbed in such form from the gastrointestinal tract or if generated in body cells from the degradation of flavoenzymes such as succinate dehydrogenase and monoamine oxidase [1].

Urinary excretion of riboflavin may be noticeable a couple of hours following oral ingestion of the vitamin. Riboflavin is a fluorescent yellow compound. Thus, following riboflavin intake in a quantity such as 1.7 mg (similar to that found in a vitamin pill), the urine changes color, deepening from a typical light yellow to a brighter orangish yellow.

RECOMMENDED DIETARY ALLOWANCE

The RDAs for riboflavin have been estimated through various studies involving urinary excretion of riboflavin, the relationship of dietary intake to clinical signs of deficiency, and the activity of erythrocyte glutathione reductase. The latest (1998) recommendations for riboflavin are similar to the 1989 RDA, which, for adults, suggested a minimum intake of 1.2 mg for persons whose caloric intake may be <2,000 kcal [14]. The current RDAs for riboflavin for adult men and women are 1.3 mg/day and 1.1 mg/day, respectively; the estimated average requirements for adult men and women are 1.1 mg and 0.9 mg, respectively [1]. With pregnancy and lactation, recommendations for daily riboflavin intake increase to 1.4 mg and 1.6 mg, respectively [1]. The inside covers of the book provide additional RDAs for riboflavin for other age groups.

DEFICIENCY: ARIBOFLAVINOSIS

A deficiency of riboflavin, known as ariboflavinosis, rarely occurs in isolation but most often is accompanied by

other nutrient deficits. No clear riboflavin deficiency disease has been characterized; however, clinical symptoms of deficiency after almost 4 months of inadequate intake include lesions on the outside of the lips (cheilosis) and corners of the mouth (angular stomatitis), inflammation of the tongue (glossitis), redness or bloody (hyperemia) and swollen (edema) mouth/oral cavity, an inflammatory skin condition seborrheic dermatitis, anemia, and peripheral nerve dysfunction (neuropathy), among other signs. Severe deficiency of riboflavin may diminish the synthesis of the coenzyme form of vitamin B₆ and the synthesis of niacin (NAD) from tryptophan. Studies in cell cultures have found that riboflavin deficiency can result in protein and DNA damage and arrest cells in the G1 phase of the cell cycle [13].

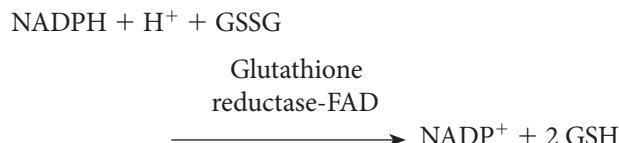
Because of limited dietary intake, people with congenital heart disease, some cancers, and excess alcohol intake may develop deficiency. The deficiency condition also remains fairly common in developing countries such as India [15]. Riboflavin metabolism is altered with thyroid disease. Excretion of riboflavin is enhanced with diabetes mellitus, trauma, and stress. Women on oral contraceptives are more likely to develop deficiency than women not taking these drugs. In addition, it has been shown that poor riboflavin status in people who have a homozygous mutation (677C → T) in methylene tetrahydrofolate reductase further increases plasma homocysteine concentrations, a risk factor for heart disease [16,17]; see the Perspective at the end of this chapter for a further discussion of this enzymatic mutation.

TOXICITY

Toxicity associated with large oral doses of riboflavin has not been reported, and no tolerable upper intake level for riboflavin has been established [1]. Trials have shown use of large amounts (400 mg) of the vitamin to be effective in treating migraine headaches without side effects [18,19].

ASSESSMENT OF NUTRITION

The most sensitive method for determining riboflavin nutriture is to measure the activity of erythrocyte glutathione reductase, an enzyme requiring FAD as a coenzyme. The method is based on the following reaction:



Glutathione in its oxidized form is designated GSSG, and in its reduced form GSH. In cases of a riboflavin deficiency

or marginal riboflavin status, the activity of glutathione reductase is limited, and less NADPH is used to reduce the oxidized glutathione. In vitro enzyme activity in terms of “activity coefficients” (AC) is determined both with and without the addition of FAD to the medium. Activity coefficients represent a ratio of the enzyme’s activity with FAD to the enzyme’s activity without FAD. When the addition of FAD stimulates enzyme activity to generate an AC of 1.2 to 1.4, riboflavin status is considered low; an AC > 1.4 suggests riboflavin deficiency. Conversely, if FAD is added and AC is < 1.2, then riboflavin status is considered acceptable.

Cellular riboflavin concentrations and urinary riboflavin excretion also are used to assess status. Cellular riboflavin concentrations < 10 µg/dL and urinary riboflavin excretion < 19 µg/g creatinine (without recent riboflavin intake) or < 40 µg per day are indicative of deficiency.

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Niacin (Vitamin B₃)

The term *niacin* (vitamin B₃) is considered a generic term for nicotinic acid and nicotinamide (also called niacinamide). The vitamin was once called the anti-black tongue factor because of its effect in dogs. The vitamin activity of niacin is provided by both nicotinic acid and nicotinamide. Structurally, nicotinic acid is pyridine 3-carboxylic acid, whereas nicotinamide is nicotinic acid amide (Figure 9.13).

Like thiamin, which was discovered through its deficiency disorder beriberi, niacin was discovered through the condition pellagra in humans and a similar condition, called black tongue, in dogs. Pellagra was especially prevalent in the Southern United States where corn (which contains a relatively unavailable form of niacin) was a main dietary staple in the early 1900s. It was not until about 1937 that Elvehjem isolated the vitamin, which was shown then to cure both pellagra and black tongue.

SOURCES

The best sources of niacin include fish such as tuna and halibut, and meats such as beef, chicken, turkey, and pork, among others. Enriched cereals and bread products, whole grains, fortified cereals, seeds, and legumes also contain appreciable amounts of niacin. Niacin is also found in coffee and tea, and in lesser amounts in green vegetables and milk. In supplements, niacin is generally found as nicotinamide (niacinamide).

In animals, niacin occurs mainly as the nicotinamide nucleotides, that is, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). However, following the slaughter of animals, NAD and NADP are thought to undergo hydrolysis; thus, meats provide niacin as free nicotinamide. Figure 9.14 shows the structures of NAD and NADP. In their oxidized forms, NAD and NADP possess a positive charge and therefore may alternatively be written NAD⁺ and NADP⁺.

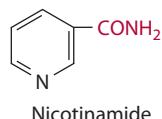


Figure 9.13 Nicotinic acid and nicotinamide.

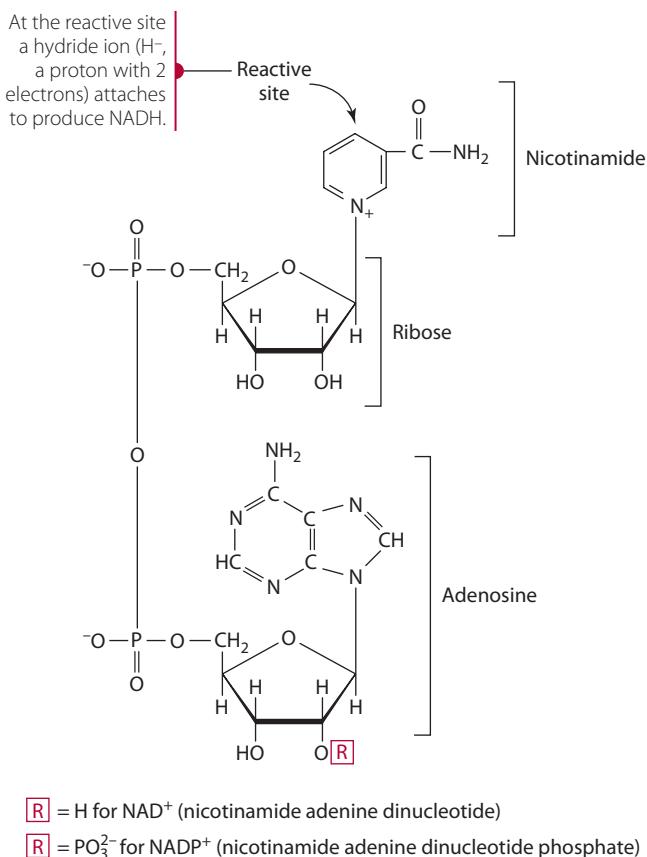


Figure 9.14 The structures of NAD and NADP.

In some foods, niacin may be bound covalently to complex carbohydrates and called niacytin, or it may be bound to small peptides and called niacinogens. This bound form of niacin is found primarily in corn but also in wheat and some other cereal products. Chemical treatment with bases such as lime water can improve availability of some bound niacin. Some niacin also may be released from niacytin on exposure to gastric acid. However, only about 10% of the niacin from maize is thought to be available for absorption.

In addition to dietary sources of niacin, NAD may be synthesized in the liver from the amino acid tryptophan. This biosynthetic pathway, which provides an important contribution to the niacin needs of the body, is depicted in Figure 9.15. Only about 3% of the tryptophan that is metabolized follows the pathway to NAD synthesis. An estimated 1 mg niacin is produced from ingestion of 60 mg dietary tryptophan (see the RDA section for niacin to understand how this synthesis is accounted for in niacin recommendations). Riboflavin (FAD), vitamin B₆ (PLP), and iron are required in some of the reactions involved in the conversion of tryptophan to NAD. In fact, deficiency of these nutrients, along with other dietary factors such as poor tryptophan and energy intakes, can impair NAD synthesis.

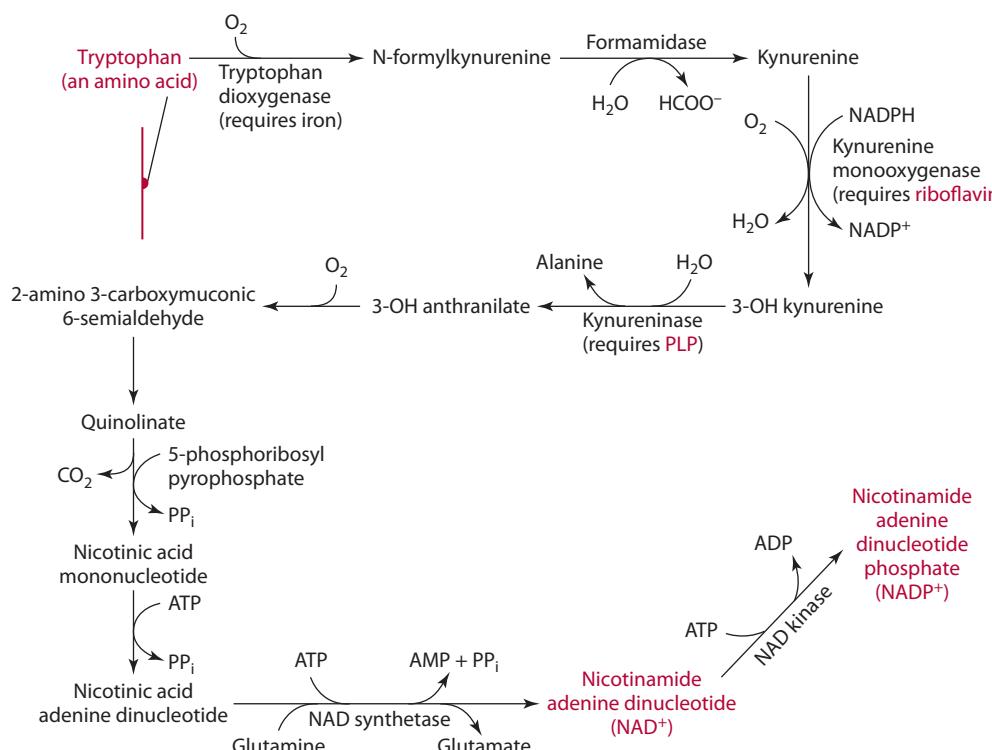
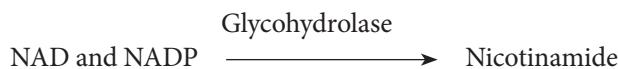


Figure 9.15 NAD⁺ and NADP⁺ synthesis from the amino acid tryptophan.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

NAD and NADP may be hydrolyzed within the intestinal tract or enterocyte by glycohydrolase to release free niacinamide. A pyrophosphatase also is involved in phosphate hydrolysis.



Nicotinamide and nicotinic acid can be absorbed in the stomach, but they are more readily absorbed in the small intestine [1].

In the small intestine, niacin, if present in low concentrations, is absorbed by sodium-dependent, carrier-mediated (facilitated) diffusion. At high concentrations (as with 3–4 g pharmacological doses), niacin is absorbed almost completely by passive diffusion.

In the plasma, niacin is found primarily as niacinamide, but nicotinic acid also may be found. Up to about one-third of nicotinic acid in the plasma is bound to plasma proteins. From the blood, niacinamide and nicotinic acid move across cell membranes by simple diffusion; however, nicotinic acid transport into the kidney tubules and red blood cells requires a carrier.

Nicotinamide serves as the primary precursor of NAD, which is synthesized in all tissues. However, nicotinic acid in the liver also may be used to synthesize NAD. In the liver, NAD synthesis from niacinamide appears to be influenced by various hormones.

As NAD or NADP, the vitamin is trapped within the cell. Intracellular concentrations of NAD typically predominate over those of NADP. In the liver, excess niacin and tryptophan are converted to NAD, which is stored in small amounts not bound to enzymes. NAD may be degraded to yield niacinamide, which then is available for transport to other tissues. The coenzyme forms of niacin are the ones that function in the body. NAD is found primarily in its oxidized form (NAD), whereas NADP is found in cells mainly in its reduced form (NADPH).

FUNCTIONS AND MECHANISMS OF ACTION

Approximately 200 enzymes, primarily dehydrogenases, require the coenzymes NAD and NADP, which act as a hydrogen donor or electron acceptor. Figure 9.16 demonstrates the oxidation-reduction that may occur in the niacinamide moiety of the coenzymes. In addition to its coenzyme roles, niacin functions as a substrate in non-redox roles as a donor of adenosine diphosphate ribose (ADP-ribose).

Coenzymes

Although NAD and NADP are very similar and undergo reversible reduction in the same way, their functions in the cell are quite different. The major role of NADH, formed from NAD, is to transfer its electrons from metabolic intermediates through the electron transport chain (Figure 3.26), thereby producing adenosine triphosphate (ATP). NADPH, in contrast, acts as a reducing agent in many biosynthetic

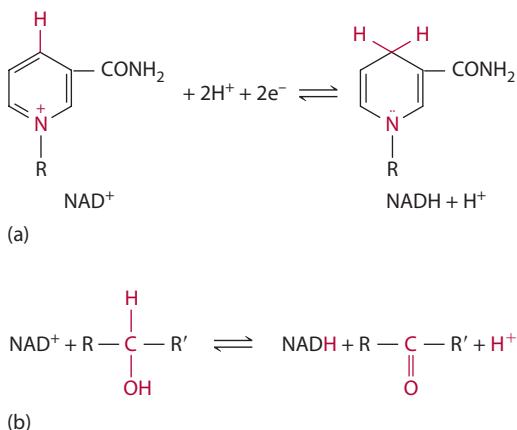


Figure 9.16 (a) The oxidation and reduction in the nicotinamide moiety. (b) The role of NAD in dehydrogenation reactions. One H of the substrate goes to NAD.

pathways such as fatty acid, cholesterol, and steroid hormone synthesis and also in other pathways. NAD and NADP coenzymes are tightly bound to their apoenzymes and can easily transport hydrogen atoms from one part of the cell to another. Reactions in which they participate occur both in the mitochondria and in the cytoplasm.

Oxidative reactions in which NAD participates and is reduced to NADH include:

- glycolysis (Figure 3.17)
- oxidative decarboxylation of pyruvate (Figure 9.9)
- oxidation of acetyl CoA in the TCA cycle (Figure 3.19)
- β-oxidation of fatty acids (Figure 5.23)
- oxidation of ethanol (Figure 5.35)

NAD is also required by aldehyde dehydrogenase for catabolism of vitamin B₆ as pyridoxal to its excretory product, pyridoxic acid.

NADPH is generated from NADP by reduction. This reaction occurs as part of the hexose monophosphate shunt (Figure 3.31) and by the mitochondrial membrane malate aspartate shuttle (Figure 3.22). The NADPH produced in these reactions is used in a variety of reductive biosyntheses, including:

- fatty acid synthesis (Figure 5.29)
- cholesterol and steroid hormone synthesis
- oxidation of glutamate (Figure 6.35)
- synthesis of deoxyribonucleotides (precursors of DNA)
- regeneration of glutathione, vitamin C, and thioredoxin

Several reactions in folate metabolism also are dependent on NADPH. For example, conversion of folate to dihydrofolate (DHF) and tetrahydrofolate (THF) and synthesis of 5-methyl THF and 5,10-methylene THF, active forms of folate, require NADPH (see Figure 9.28 in the folate section of this chapter).

Nonredox Roles

NAD acts as a donor of adenosine diphosphate ribose (ADP-ribose) for the posttranslational modification of proteins as well as a donor of ADP-ribose for the formation of cyclic ADP-ribose. Mono ADP-ribosyl transferases (sometimes abbreviated ARTs) transfer one (mono) ADP-ribose from NAD to various acceptor proteins found on cell surfaces or outside cells. For example, the substrate for ART 1 is defensin, an antimicrobial peptide made by immune system cells and thus important in the immune response [2]. Other mono ADP-ribosyl transferases are found within cells or attached to cell membranes and function to modify proteins typically involved in cell regulation of the cell cytoskeleton and cell signalling [2]. Poly ADP-ribose polymerases (PARP) transfer several (*poly* >200) polymers of branched ADP-riboses from NAD onto various target proteins. Targets for these intracellular transferases include, for example, various chromosomal proteins, including both histone and nonhistone proteins. The proteins are thought to function in the nucleus in DNA repair and replication as well as in cell differentiation [2,3]. Cyclic ADP-ribose, generated by NAD glycohydrolases, is thought to function in certain cells as a second messenger involved in control of ryanodine receptors and in mobilization of calcium from intracellular stores [4–6].

METABOLISM AND EXCRETION

NAD, generated from nicotinamide or produced in the liver from tryptophan, and NADP can be degraded by glycohydrolase into nicotinamide and ADP-ribose. The released nicotinamide is methylated and is then oxidized in the liver into a variety of products that are excreted in the urine. Typically, little nicotinic acid or nicotinamide is excreted, because both compounds may be actively reabsorbed from glomerular filtrate.

The primary metabolites of nicotinamide are N' methyl nicotinamide (sometimes abbreviated NMN and representing ~20% to 30% of niacin metabolites) and N' methyl 2-pyridone 5-carboxamide (also called 2-pyridone and representing ~40% to 60%). Small amounts of N' methyl 4-pyridone carboxamide (called 4-pyridone) also may be present. Nicotinic acid is metabolized mainly to N' methylnicotinic acid.

RECOMMENDED DIETARY ALLOWANCE

Recommendations for niacin intake include calculations of niacin derived from the amino acid tryptophan, with about 60 mg of tryptophan thought to generate 1 mg of niacin. Total niacin thus is provided to the body as nicotinic acid and nicotinamide and from 1/60 mg of tryptophan. The term *niacin equivalent* (NE) is used to account for the provision by tryptophan.

Although recommendations are given in niacin equivalents, food composition tables report only preformed niacin. A rough estimate of niacin equivalents from a protein can be made by assuming that 10 mg of tryptophan are provided for every 1 g of high-quality (complete) protein in the diet; that is, 1 g of complete, high-quality protein = 10 mg of tryptophan. This estimate means that an intake of 60 g of complete protein, for example, would provide 600 mg tryptophan: (10 mg tryptophan/1 g protein × 60 g protein = 600 mg tryptophan). Then, because it takes 60 mg of tryptophan to generate 1 mg of NE, 60 g of protein would generate about 10 NEs (600 mg of tryptophan × 1 mg of NE/60 mg of tryptophan = 10 NEs). The average U.S. diet usually contains about 900 mg tryptophan daily [7].

Information used in estimating niacin requirements and recommendations has come from several studies, including human depletion and repletion studies as well as other studies with primarily urinary metabolites of niacin serving as indicators to base requirements and recommendations. The RDAs for niacin (as niacin equivalents) for adult men and women are 16 mg/day and 14 mg/day, respectively [8]. Estimated requirements are 12 mg and 11 mg niacin for adult men and women, respectively. With pregnancy and lactation, the RDA for niacin increases to 18 mg and 17 mg niacin equivalents, respectively [8]. The inside covers of the book provide additional RDAs for niacin for other age groups.

DEFICIENCY: PELLAGRA

Classical deficiency of niacin results in a condition known as **pellagra**. The four Ds—*dermatitis, dementia, diarrhea, and death*—are often used as a mnemonic device for remembering signs of pellagra [9]. The dermatitis is similar to sunburn at first and appears on areas exposed to sun, such as the face and neck, and on extremities such as the back of the hands, wrists, elbows, knees, and feet. Neurological manifestations include headache, apathy, loss of memory, peripheral neuritis, paralysis of extremities, and dementia or delirium. Gastrointestinal manifestations include glossitis, cheilosis, stomatitis, nausea, vomiting, and diarrhea or constipation. If untreated, death occurs.

A niacin deficiency or diminished niacin status also can result from the use of some medications and from some disorders such as malabsorptive conditions. The antituberculosis drug isoniazid, for example, binds with vitamin B₆ as PLP and thereby reduces PLP-dependent kynureninase activity required for niacin synthesis. Malabsorptive disorders (chronic diarrhea, inflammatory bowel diseases, some cancers) may impair niacin and tryptophan absorption and result in the increased likelihood of niacin deficiency. Hartnup disease results in impaired absorption of tryptophan (and other neutral amino acids), thus decreasing concentrations of the precursor tryptophan needed for

niacin synthesis. People with poor nutrient intakes, such as those who consume excessive amounts of alcohol, are at risk for niacin deficiency.

TOXICITY

Large doses of nicotinic acid (up to 6 g/day in divided doses) are used to treat hypercholesterolemia (high blood cholesterol). These pharmacological doses have been shown to significantly lower total serum cholesterol, triglycerides, and low-density lipoproteins (LDLs) and increase high-density lipoproteins (HDLs). Although the mechanisms of action are not fully understood, niacin appears to act in multiple ways to improve serum lipids. Niacin (when given in pharmacologic doses) inhibits lipolysis in adipose tissue and decreases hepatic VLDL secretion from the liver and LDL production. Niacin also inhibits diacylglycerol acyltransferase in the liver to diminish triglyceride synthesis and by other mechanisms (as reviewed by Ganji [10]) increases HDL cholesterol concentrations [10–12]. Niacin appears to act (in part) by binding to selected G-protein-coupled receptors such as HM74A. This receptor on adipose tissue, for example, binds to niacin with high affinity and mediates an inhibitory G-protein signal that reduces cyclic AMP production by adenyl cyclase and also reduces hormone-sensitive lipase activity caused by decreased activation of protein kinase A [11,13,14]. Niacin also induces peroxisome proliferator-activated receptor gamma (PPAR γ) expression and transcriptional activation in macrophages through HM74a-mediated stimulation of prostaglandin synthesis [13].

Despite the therapeutic benefits of nicotinic acid, some undesirable side effects are associated with its use as a drug, especially in certain forms and in doses of 1 g or more per day. Some of these side effects include:

- vasodilatory effects, mediated in part by release of histamine, including uncomfortable flushing and redness along with burning, itching (pruritus), tingling, and headaches
- gastrointestinal problems such as heartburn, nausea, and possibly vomiting
- liver injury (hepatic toxicity), indicated by elevated serum levels of enzymes of hepatic origin (e.g., transaminases and alkaline phosphatases), jaundice secondary to obstructed bile flow from the liver to the small intestine, hepatitis, and liver failure
- hyperuricemia and possibly gout, because niacin competes with uric acid for excretion, thus raising serum uric acid levels
- elevation of plasma glucose concentrations (i.e., glucose intolerance) [15–20]

Newer extended-release forms of nicotinic acid are available with fewer side effects. Nicotinamide in large doses does not exhibit toxic effects, but neither does it reduce blood lipids.

Because of the vasodilatory effects associated with nicotinic acid consumption from supplements, a tolerable upper intake level for adults for niacin (both nicotinic acid and niacinamide) from supplements and from fortified foods [8] has been set at 35 mg/day. Use of nicotinic acid as a cholesterol-lowering agent must be weighed against its potential toxic effects for those using the vitamin to treat hyperlipidemia.

ASSESSMENT OF NUTRITION

Several methods have been employed to assess niacin status. Most methods involve the measuring one or more urinary metabolites of the vitamin. Urinary excretion of <0.8 mg/day of N' methyl niacinamide is considered suggestive of niacin deficiency. Urinary excretion of <0.5 mg N' methyl niacinamide/1 g creatinine also has been suggested as indicative of poor (deficient) niacin status [21]. Marginal niacin status is suggested by urinary amounts in the range of 0.5 to 1.59 mg N' methyl niacinamide/1 g creatinine, and levels in excess of 1.6 suggest adequate status [21]. This ratio, however, has been criticized as being difficult to interpret because of multiple influences on urinary creatinine excretion. It usually is employed during a period of 4 to 5 hours after a 50 mg test dose of niacinamide. Another ratio employed to assess niacin status is that of urinary N' methyl 2-pyridone 5-carboxamide (2-pyridone) to N' methyl niacinamide (NMN). Although a ratio of <1 is found with niacin deficiency, this ratio is not thought to be sensitive enough to detect marginal intakes of niacin and may better reflect dietary protein adequacy as opposed to niacin status [22].

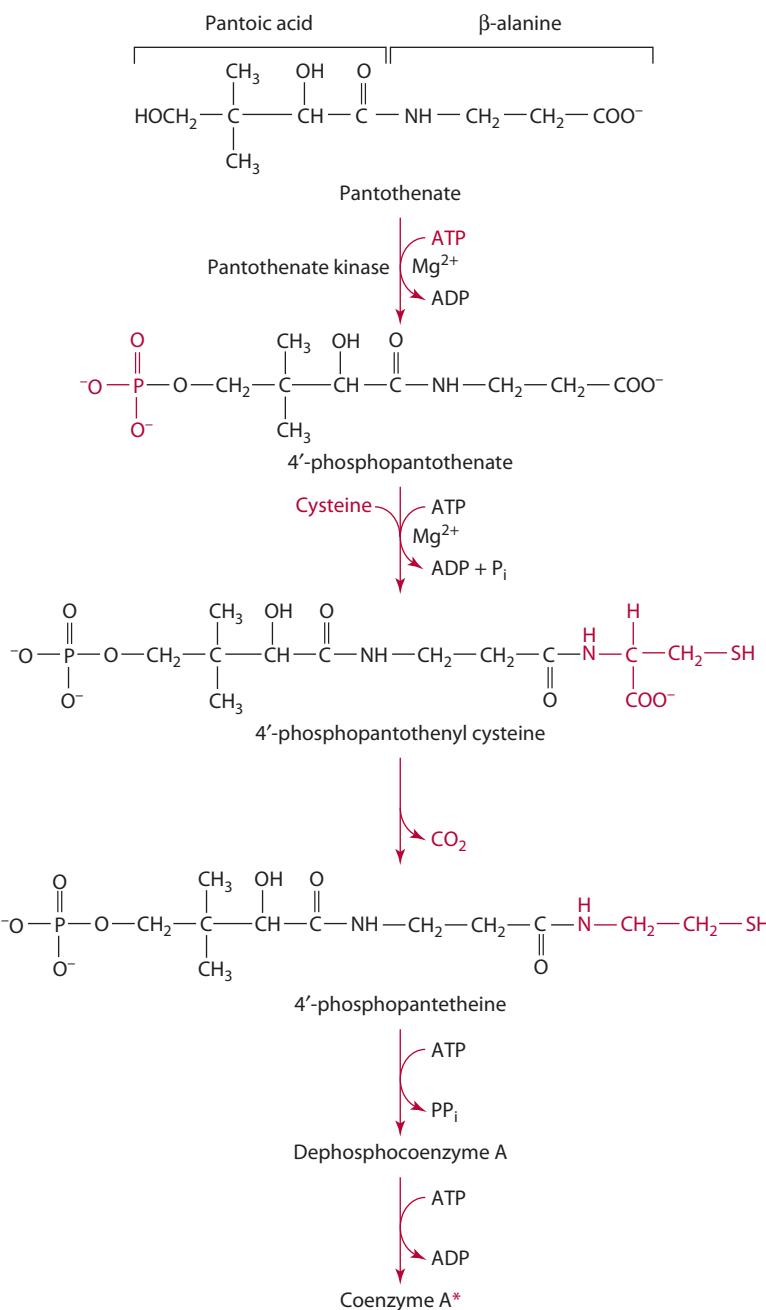
In addition to measurement of urinary metabolites, erythrocyte NAD concentrations and the ratio of NAD to NADP (<1.0) have been used to assess niacin status [23, 24]. Plasma concentrations of 2-pyridone have been shown to drop below the level of detection with low niacin intakes and thus may be used as an index of niacin status [23].

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Pantothenic Acid

Pantothenic acid consists of β -alanine and pantoic acid joined by a peptide bond/amide linkage. The structure of pantothenate is shown at the top of Figure 9.17 and as part of coenzyme A (the A referring to acetylation) in Figure 9.18. The vitamin was once called vitamin B₅. Pantothenic acid's essentiality was not discovered until 1954, although the vitamin had been isolated in about 1931 by R. J. Williams and its structure determined in 1939. It was F. Lipmann, who later (1957) won the Nobel prize for his work, who showed that coenzyme A facilitated biological acetylation reactions.



*Structure shown in Fig. 9.18

Figure 9.17 Synthesis of coenzyme A from pantothenate.

SOURCES

The Greek word *pantos* means “everywhere,” and the vitamin pantothenic acid, as its name implies, is found widely distributed in nature. Because this vitamin is present in virtually all plant and animal foods, a deficiency is quite unlikely. Meats (particularly liver), egg yolk, legumes, whole-grain cereals, potatoes, mushrooms, broccoli, and avocados, among other foods, are good sources of the vitamin. Royal jelly from bees also provides large amounts of pantothenate. In supplements, pantothenate is usually found as calcium pantothenate

or as panthenol, an alcohol form of the vitamin. Most adults in the United States consume about 4 to 7 mg pantothenic acid per day.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Pantothenic acid occurs in foods in free and bound forms. About 85% of the pantothenic acid in food occurs bound as a component of coenzyme A, abbreviated CoA. During the digestive process, CoA is hydrolyzed in the lumen

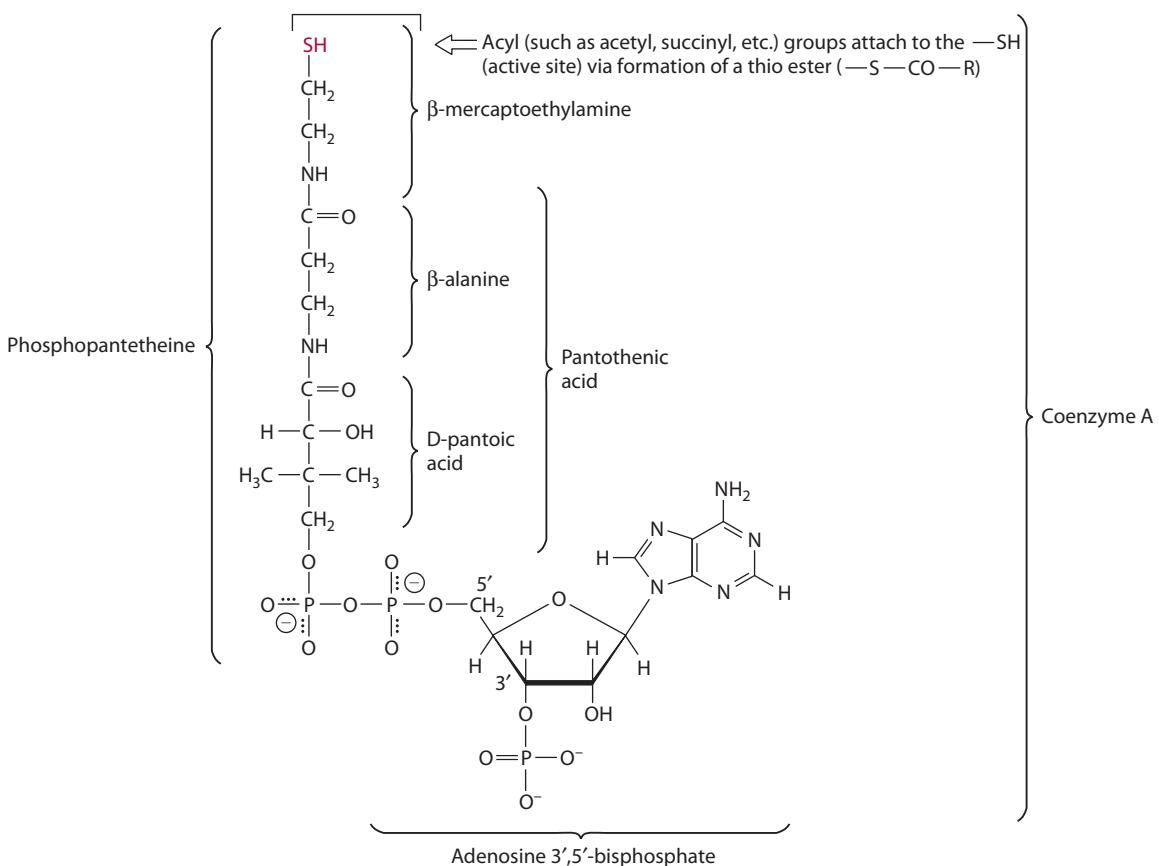


Figure 9.18 Structure of coenzyme A and identification of components.

in several steps to pantothenic acid by phosphatases and pyrophosphatases.

Pantothenic acid is thought to be absorbed principally in the jejunum by passive diffusion when present in high concentrations and by a sodium-dependent active multivitamin transporter/carrier (SMVT) when present in low concentrations. Pantothenic acid shares this common intestinal multivitamin transporter/carrier with biotin (another B vitamin) and lipoic acid [1]. Approximately 40% to 61%, mean 50%, of the ingested pantothenic acid is absorbed [2,3]. Panthenol, the alcohol form of the vitamin used in multivitamins, is also absorbed and converted to pantothenate. However, pantothenic acid absorption has been shown to decrease to about 10% when vitamin ingestion approaches 10 times the recommended intake in pill form.

From the intestinal cell, pantothenic acid enters portal blood for transport to body cells. Pantothenic acid is found free in blood plasma/serum; however, higher concentrations are found intracellularly (specifically within red blood cells) than extracellularly (in plasma/serum) [4].

Uptake of pantothenic acid by tissues differs. The uptake of pantothenic acid by tissues such as the heart, muscle, brain, and liver cells occurs by sodium-dependent active transport, whereas uptake by other tissues occurs by facilitated diffusion [4–7]. Within cells, pantothenic acid along with

4-phosphopantothenate and pantetheine may be found. Most pantothenic acid is used to synthesize or resynthesize CoA [4], which is found in fairly high concentrations in the liver, adrenal gland, kidney, brain, and heart [5–7].

FUNCTIONS AND MECHANISMS OF ACTION

Pantothenic acid functions in the body as a component of CoA and 4'-phosphopantetheine. The synthesis of 4'-phosphopantetheine and CoA from pantothenate is depicted in Figure 9.17. Overall, the synthesis requires pantothenic acid, the amino acid cysteine, and ATP.

- The synthesis of coenzyme A starts with the rate-limiting phosphorylation of pantothenic acid by pantothenate kinase and the formation of 4'-phosphopantothenate. ATP and Mg²⁺ are required for this reaction.
- Next, in another ATP- and Mg²⁺-requiring reaction, cysteine reacts with the 4'-phosphopantothenate. A peptide bond is formed between the carboxyl group of the 4'-phosphopantothenate and the amino group of cysteine.
- Third, a carboxyl group from the cysteine moiety is removed to generate 4'-phosphopantetheine.
- Next, an adenylation occurs whereby ATP reacts with the 4'-phosphopantetheine; adenosine monophosphate

(AMP) is added to the 4'-phosphopantetheine to form dephosphocoenzyme A with the release of pyrophosphate.

- Last, phosphorylation with ATP of the 3'-hydroxyl group of the dephosphocoenzyme A produces CoA.

The synthesis of CoA is inhibited by acetyl CoA, malonyl CoA, and propionyl CoA as well as by other, longer-chain acyl CoAs. CoA metabolism has been reviewed in depth by Robishaw and Neely [8]. Figure 9.18 shows the structure of CoA. Note from this figure that CoA contains several components, including phosphopantetheine and adenosine 3',5'-bisphosphate. The figure also identifies the active site where CoA binds to acyl groups.

The compound 4'-phosphopantetheine, as part of the acyl carrier protein complex, and CoA function as carriers or transporters of acetyl and acyl groups. The vitamin forms

thio esters $\text{O} \parallel \text{S}-\text{C}-\text{R}$ with carboxylic acids and can transfer the acetyl or acyl groups, typically 2 to 13 carbons, as needed for various cellular reactions. Examples of carboxylic acids carried by CoA include:

- acetic (two carbons)
- malonic (three carbons)
- propionic (three carbons)
- methylmalonic (four carbons)
- succinic (four carbons)

These carboxylic acids arise in the body during metabolism, and some can be obtained exogenously by ingesting food. For example, propionic acid is found naturally in some fish and also is derived from the catabolism of several amino acids, including methionine, threonine, and isoleucine, and from the catabolism of odd-chain fatty acids. As another example, succinate is found as an intermediate in the TCA cycle.

Pantothenic acid, as part of CoA and 4'-phosphopantetheine, participates extensively in nutrient metabolism, including degradation reactions resulting in energy production and synthetic reactions for the production of many vital compounds. In addition to its role in nutrient metabolism, CoA acetylates nutrients including sugars and proteins among others. Some of the specific reactions and processes involving CoA and 4'-phosphopantetheine are presented next.

The metabolism of carbohydrate, lipids, and protein (energy-producing nutrients) relies to varying degrees on CoA. For example, a crucial reaction in nutrient metabolism is the conversion of pyruvate to acetyl CoA, which condenses with oxaloacetate to introduce acetate for oxidation during the TCA cycle (Figure 9.8). Acetyl CoA, the common compound formed from the three energy-producing nutrients, holds the central position in the transformation of energy. Pantothenic acid thus joins the B vitamins thiamin, riboflavin, and niacin in the oxidative

decarboxylation of pyruvate (Figure 9.9). These same vitamins also participate in the oxidative decarboxylation of α -ketoglutarate to succinyl CoA, a TCA cycle intermediate and compound used with the amino acid glycine to synthesize heme (Figure 12.5).

In lipid metabolism, CoA is important in the synthesis of cholesterol, bile salts, ketone bodies, fatty acids, and steroid hormones. For example, in cholesterol and ketone body synthesis, acetyl CoA and acetoacetyl CoA react to form the key intermediate HMG CoA (Figure 5.33). Condensation of acetyl CoA with activated CO_2 to form malonyl CoA represents the first step in fatty acid synthesis (Figure 5.27). Moreover, phospholipid and sphingomyelin production from phosphatidic acid and sphingosine, respectively, also use acyl CoA.

Pantothenic acid as 4'-phosphopantetheine also functions as the prosthetic group for acyl carrier protein (ACP). Acyl carrier protein acts as the acyl carrier in the synthesis of fatty acids and is a necessary component of the fatty acid synthase complex. The sulphydryl group in the 4'-phosphopantetheine and a sulphydryl group in the protein are the active sites in the acyl carrier protein. These two groups are located close to each other so the acyl chain being synthesized can be transferred between them.

Pantothenic acid as CoA is involved in the acetylation (donation of the long-chain fatty acids or acetate) of some proteins and sugars as well as some drugs. The acetylation of the proteins by CoA occurs posttranslationally and in turn affects protein functions [9,10]. For example, acetylation of some proteins and peptides prolongs half-life, thereby delaying the degradation of the protein. Acetylation of the N-terminal amino acids of some proteins has been shown to affect resistance to ubiquitin-mediated proteolysis [9]. Acetylation also affects the activity, location, and function of proteins in cells [9,10]. Acetylation of some enzymes, for example, results in either activation or inactivation. Other proteins that may undergo acetylation include microtubules of the cell's cytoskeleton as well as histones and other DNA-binding proteins. Microtubules, made from polymerization of α - and β -tubulin dimers, appear to be stabilized by acetylation and destabilized when deacetylated. Choline is acetylated to form the neurotransmitter acetylcholine. Also, aminosugars such as glucosamine and galactosamine are acetylated by CoA to form N-acetyl glucosamine and N-acetyl galactosamine, respectively. These acetylated aminosugars in turn may function structurally in the cell, for example, to provide recognition sites on cell surfaces or to direct proteins for membrane functions, among other roles.

A yet unidentified role of pantothenic acid, based on animal studies, involves healing. The vitamin appears to accelerate the normal healing process following surgery [11]. The exact mechanism by which pantothenic acid improves healing is unclear; however, an increase in postoperative cellular multiplication has been proposed [11].

METABOLISM AND EXCRETION

Pantothenic acid does not appear to undergo metabolism prior to excretion. Pantothenic acid is excreted intact primarily in the urine, with only small amounts excreted in the feces. No metabolites of the vitamin have been identified in the urine or feces. Urinary excretion of the vitamin usually ranges from about 2 to 7 mg/day.

ADEQUATE INTAKE

The adequate intake (AI) recommendation for adults age 19 years and older for pantothenic acid has been set at 5 mg [3]. Adequate intake is used instead of RDA when insufficient data are available to establish an estimated average requirement (EAR) and subsequent RDA [3]. AIs for pantothenic acid of 6 mg/day and 7 mg/day are suggested for women during pregnancy and lactation, respectively [3]. The inside covers of this book provide AIs for pantothenic acid for other age groups.

DEFICIENCY: BURNING FEET SYNDROME

“Burning feet syndrome” is characterized by numbness of the toes and a sensation of burning in the feet. The condition is exacerbated by warmth and diminished with cold and is thought to result from pantothenic acid deficiency. The syndrome can be corrected with calcium pantothenate administration. Other symptoms of deficiency include vomiting, fatigue, weakness, restlessness, and irritability. A metabolic inhibitor of pantothenate, omega methylpantothenate, has been used in studies to induce low pantothenate status in humans.

Deficiency of pantothenic acid is thought to occur more often in conjunction with multiple nutrient deficiencies, as for example in malnutrition. Some conditions that may increase the need for the vitamin include alcoholism, diabetes mellitus, and inflammatory bowel diseases. Increased excretion of the vitamin has been shown in people with diabetes mellitus. Absorption is likely to be impaired with inflammatory bowel diseases. Intake of the vitamin typically is low in people with excessive alcohol intake.

TOXICITY

Pantothenate toxicity has not been reported to date in humans. Intakes of about 10 g pantothenate as calcium pantothenate daily for up to 6 weeks have resulted in no problems [3]. However, intakes of about 15 to 20 g have been associated with mild intestinal distress and diarrhea, and lower intakes of 100 mg may increase niacin excretion [3,12].

ASSESSMENT OF NUTRITION

Blood pantothenic acid concentrations <100 mg/dL are thought to reflect low dietary pantothenate intakes; however, blood concentrations do not correlate well with changes in dietary pantothenate intake and status [13,14]. Urinary pantothenate excretion is considered to be a better indicator of status, with excretion of <1 mg/day considered indicative of poor status.

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Biotin

Biotin consists structurally of two rings—a ureido ring joined to a thiophene ring—with an additional valeric acid side chain (Figure 9.19). Biotin’s structure was first determined by Kogl (from Europe) and by du Vigeaud and coworkers (from the United States) in the early 1940s, but the vitamin’s discovery occurred earlier. Biotin’s discovery

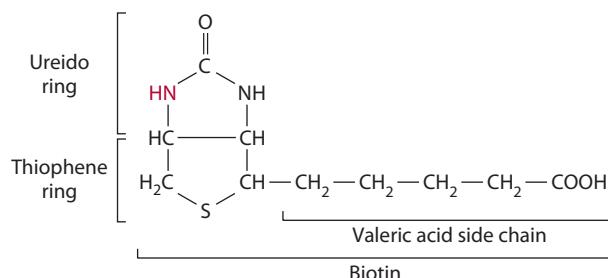


Figure 9.19 The structure of biotin.

was based on the research investigating the cause of what was called “egg white injury.” Eating raw eggs was known to result in hair loss, dermatitis, and various neuromuscular problems. Szent-Györgyi in 1931 found a substance (now called biotin) in liver that could cure and prevent the condition. Biotin was once called vitamin H (the H refers to *haut* in German and means “skin”) as well as vitamin B₇.

SOURCES

Sources of biotin for humans include biotin in dietary foods as well as biotin made by intestinal bacteria living within the large intestine. Biotin is found widely distributed in foods. Good food sources of the vitamin include liver, soybeans, and egg yolk, as well as cereals, legumes, and nuts. Within many foods, biotin is found either bound covalently to protein or as biocytin, which consists of biotin bound to the amino acid lysine. Biocytin is also sometimes called biotinyllysine (Figure 9.20).

One substance in raw egg whites, avidin, has been found to bind biotin and prevent its absorption and use by the body. Avidin is a glycoprotein and irreversibly binds biotin in what has been suggested as the tightest noncovalent bond found in nature. The binding between avidin and biotin in turn prevents biotin absorption. Yet, because avidin is heat-labile (unstable with heat), eating cooked egg whites does not compromise biotin absorption.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Protein-bound biotin requires digestion by proteolytic enzymes prior to absorption. Proteolysis by proteases yields free biotin, biocytin, or biotinyl peptides. Biotinyl peptide

can be further hydrolyzed by other proteases or peptidases within the small intestine, and biocytin can be further hydrolyzed by biotinidase. Biotinidase is found on the intestinal brush border as well as in pancreatic and intestinal juices secreted into the small intestine. The enzyme hydrolyzes the biocytin to release free biotin and lysine. Some undigested biocytin may be absorbed intact by peptide carriers and subsequently hydrolyzed by biotinidase present in plasma or in most other body tissues. Any absorbed biocytin not catabolized by biotinidase is excreted in the urine.

Biotinidase is active over a wide pH range and is specific for the biotinyl moiety [1–4]. It is found in most body cells, and its activity is expressed in multiple cellular locations including the nucleus [3,4]. At a more acidic pH, the enzyme biotinidase cleaves biocytin to produce biotin and lysine, or cleaves covalently bound biotin from any biotinyl peptides that have been released as biotinylated proteins are degraded within various cells of the body. The enzyme also cleaves biotin from histones. At a more alkaline pH, the enzyme has been shown to become biotinylated while also generating free lysine; in other words, the biotinidase enzyme becomes attached to the biotin that was previously part of biocytin. Biotinidase deficiency (first discovered in 1983) is caused by an autosomal recessive inborn error of metabolism. Should infants and children with the disorder go untreated, a biotin deficiency, among other problems, develops. Some clinical features associated with the genetic disorder include seizures, ataxia, skin rash, alopecia (hair loss), and acidosis [1,2].

Free biotin is absorbed primarily in the jejunum, followed by the ileum, because of differences in carrier concentrations [5]. Dietary biotin is thought to be nearly completely absorbed, although alcohol can inhibit biotin absorption [6]. Biotin that is synthesized by colonic bacteria is absorbed in the proximal and midtransverse colon; however, bacterially made biotin cannot totally meet the biotin needs of humans [5].

The mechanism of biotin absorption varies with intake. Biotin absorption occurs by passive diffusion with consumption of pharmacologic doses. With physiological biotin intakes, biotin absorption across the brush border membrane of the small intestine and across the colonocytes is carrier mediated and sodium dependent. The main carrier for biotin found in the small intestine as well as the

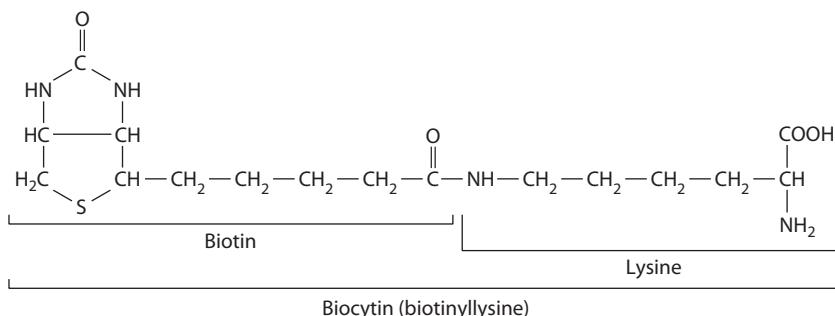


Figure 9.20 The structure of biocytin, also called biotinyllysine.

liver (among other tissues) also transports pantothenic acid and lipoic acid and is called the sodium-dependent multivitamin transporter (SMVT) [5,7,8]. Another transporter of biotin that is widely expressed in tissues is from the solute carrier gene family 19 (SLC19A3); this carrier transports both thiamin and biotin [9]. Transport of biotin across the basolateral membrane of the enterocyte is carrier mediated, but not sodium dependent [5].

Biotin is found in the plasma mostly (about 80%) in a free, unbound state, with lesser amounts bound to protein. In the blood, albumin and α - and β -globulins bind biotin, as does biotinidase, which has two binding sites for biotin and arises from hepatic secretion [10]. Biotin uptake into the liver, and probably other tissues, is thought to involve SMVT as well as other carriers. Two biotin transporters found in leukocytes include monocarboxylate transporter (MCT) 1 and another carrier from solute carrier family 19 member 3 (SLC19A3) [9,11]. Biotin is stored in small quantities in the muscle, liver, and brain [12].

FUNCTIONS AND MECHANISMS OF ACTION

Biotin functions in cells covalently bound to enzymes and thus is considered a coenzyme. In addition, biotin functions in non-coenzyme roles including possible roles in cell proliferation and gene expression.

Coenzyme Roles

For coenzyme functions within cells, biotin is attached (covalently bound) to four carboxylases. The attachment of biotin to these enzymes occurs in two steps and is cata-

lyzed by the enzyme holocarboxylase synthetase, which is found in the cytoplasm and mitochondria. In the first step of the synthesis of the carboxylases, biotin reacts with ATP in a Mg^{2+} -dependent reaction to form biotinyl adenosine monophosphate (also called activated biotin) and pyrophosphate. In the second step, the activated biotin reacts with any of four apocarboxylases to form a holoenzyme carboxylase (sometimes called holocarboxylases or just biotin-dependent carboxylases) with the release of AMP.

A mutation in holocarboxylase synthetase, as was first discovered in 1981, or a mutation in any of the four biotin-dependent carboxylases can cause problems in metabolism.

The four biotin-dependent carboxylases, which are synthesized by holocarboxylase synthetase, are acetyl CoA carboxylase, pyruvate carboxylase, propionyl CoA carboxylase, and β -methylcrotonyl CoA carboxylase. Table 9.2 lists these enzymes and their roles in metabolism. Knowles [13] provides detailed information on the mechanism of action of the biotin-dependent enzymes.

Each holoenzyme biotin-dependent carboxylase is a multisubunit enzyme to which biotin is attached by an amide linkage. Specifically, the carboxyl terminus of biotin's valeric acid side chain is linked to the ϵ amino group of a specified lysine residue in the apoenzyme [14]. The chain connecting biotin and the apoenzyme is long and flexible, allowing the biotin to move from one active site of the carboxylase to another. Figure 9.21 depicts the attachment of biotin to the enzyme, emphasizing the long, flexible chain and the amide linkage between the vitamin and the lysine residue of the enzyme. One active

Table 9.2 Biotin-Dependent Enzymes

Enzyme	Role	Significance
Pyruvate carboxylase	Converts pyruvate to oxaloacetate	Replenishes oxaloacetate for TCA cycle Necessary for gluconeogenesis
Acetyl CoA carboxylase	Forms malonyl CoA from acetate	Commits acetate units to fatty acid synthesis
Propionyl CoA carboxylase	Converts propionyl CoA to methylmalonyl CoA	Provides mechanism for metabolism of some amino acids and odd-numbered-chain fatty acids
β -methylcrotonyl CoA carboxylase	Converts β -methylcrotonyl CoA to β -methylglutaconyl CoA	Allows catabolism of leucine and certain isoprenoid compounds

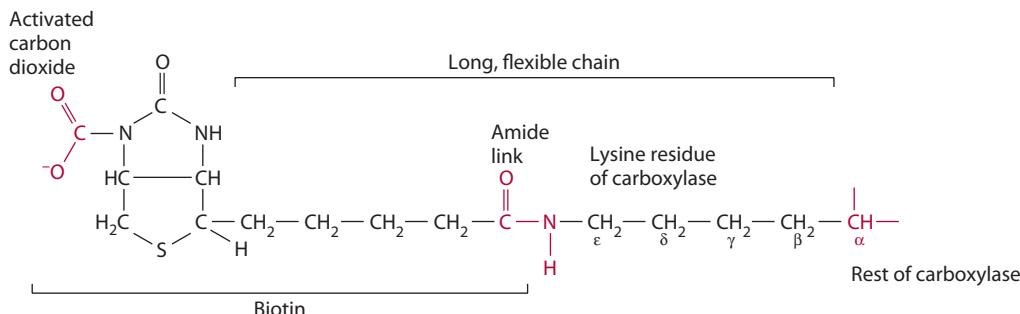


Figure 9.21 Biotin bound to the lysine residue of carboxylase and functioning as a carrier of activated CO_2 .

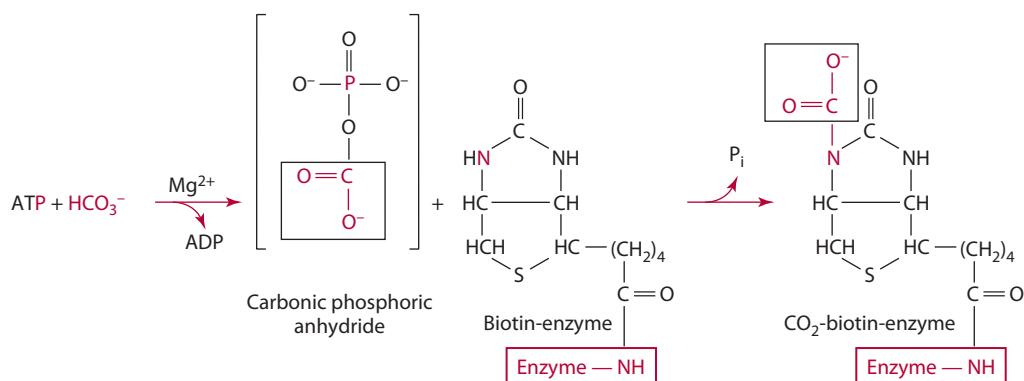


Figure 9.22 The formation of the CO_2 -biotin-enzyme complex.

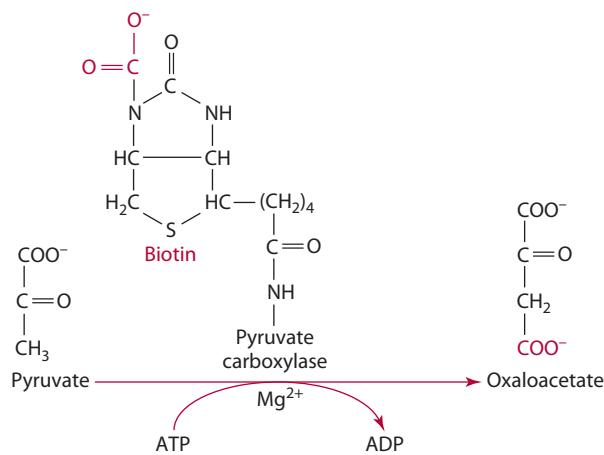


Figure 9.23 The role of biotin in the synthesis of oxaloacetate from pyruvate.

site on the apoenzyme generates the carboxybiotin enzyme, and the other transfers the activated carbon dioxide to a reactive carbon on the substrate. Figure 9.22 illustrates the formation of the CO_2 -biotin-enzyme complex.

Pyruvate Carboxylase Pyruvate carboxylase is a particularly interesting and important enzyme because of its regulatory function. Specifically, pyruvate carboxylase (a mitochondrial enzyme) catalyzes the carboxylation of pyruvate to form oxaloacetate (Figure 9.23). For its activation, pyruvate carboxylase requires the presence of acetyl CoA as well as ATP and Mg^{2+} . Acetyl CoA serves as an allosteric activator, and its presence indicates the need for increased amounts of oxaloacetate. If the cell has a surplus of ATP, the oxaloacetate is then used for gluconeogenesis. However, if the cell is deficient in ATP, the oxaloacetate enters the TCA cycle on condensation with acetyl CoA.

Acetyl CoA Carboxylase The importance of biotin in energy metabolism is further exemplified by its role in the initiation of fatty acid synthesis, that is, the formation

of malonyl CoA from acetyl CoA by the regulatory and rate-limiting enzyme acetyl CoA carboxylase. This enzyme (found in both the mitochondria and cytoplasm) is allosterically activated by citrate and isocitrate and inhibited by long-chain fatty acyl CoA derivatives. ATP and Mg^{2+} are required for the reaction (see Figure 5.27).

Propionyl CoA Carboxylase Propionyl CoA carboxylase (a mitochondrial enzyme) is important for the catabolism of the amino acids isoleucine, threonine, and methionine, each of which generates propionyl CoA. Propionyl CoA also arises from the catabolism of odd-number-chain fatty acids found, for example, in some fish. Propionyl CoA carboxylase catalyzes the carboxylation of propionyl CoA to D-methylmalonyl CoA (Figure 9.24). The reaction requires ATP and Mg^{2+} . Deficient or defective propionyl CoA carboxylase activity causes the genetic disorder propionic acidemia, characterized by the accumulation of propionyl CoA, which is then shifted into an alternate metabolic pathway. This alternate pathway results in increased production and urinary excretion of 3-hydroxypropionic acid (3HPA) and methylcitrate (MCA).

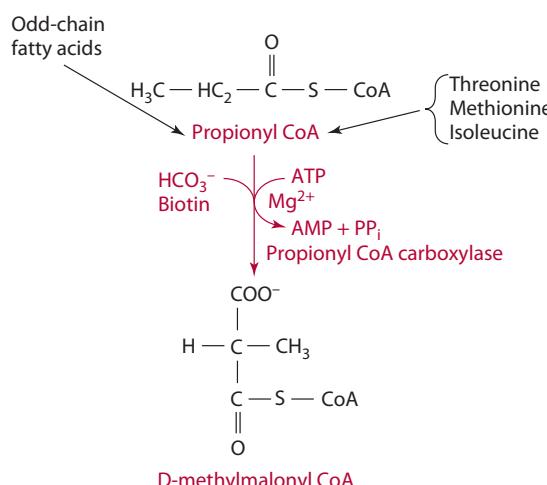


Figure 9.24 The oxidation of propionyl CoA and the role of biotin.

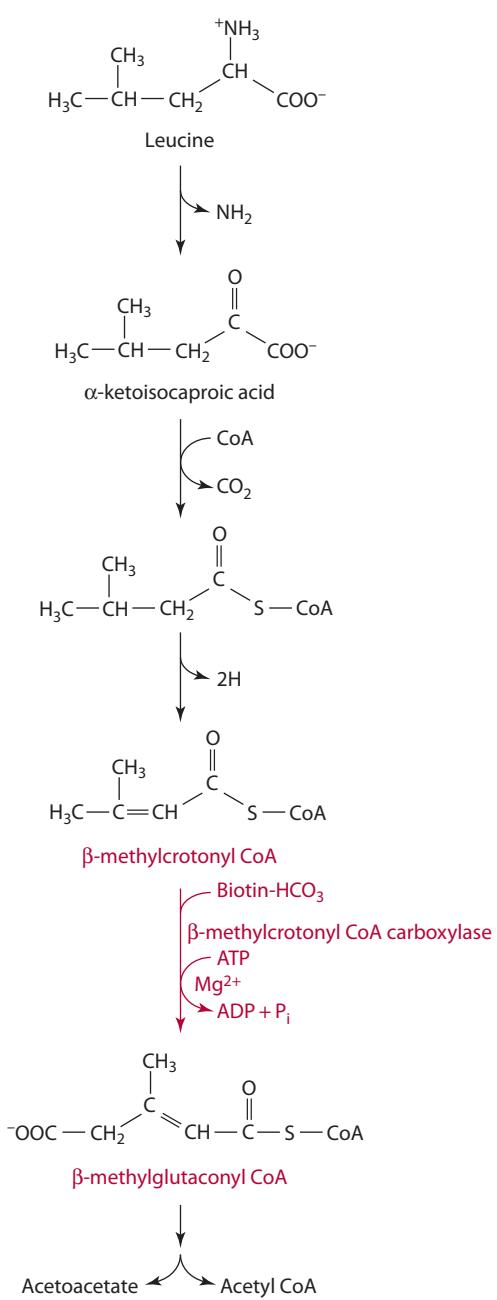


Figure 9.25 The role of biotin in leucine catabolism.

β-methylcrotonyl CoA Carboxylase β-methylcrotonyl CoA carboxylase is important in the catabolism of the amino acid leucine. During leucine catabolism (Figure 9.25), β-methylcrotonyl CoA is formed. This compound is carboxylated in an ATP-, Mg²⁺-, and biotin-dependent reaction by β-methylcrotonyl CoA carboxylase to form β-methylglutaconyl CoA, which is further catabolized to generate acetoacetate and acetyl CoA. Deficient β-methylcrotonyl CoA carboxylase activity causes accumulation of β-methylcrotonyl CoA, which is then shunted to an alternate metabolic pathway. This alternate pathway results in increased production and urinary excretion of 3-hydroxyisovaleric acid (3-HIA), 3-methylcrotonylglycine

(3MCG), and isovalerylglycine (IVG) [14,15]. Increased 3-HIA and decreased biotin concentrations in the urine are indicative of biotin deficiency [16].

Noncoenzyme Roles

Although the coenzyme role of biotin is well characterized, other possible roles of biotin are less known and investigated. Some of these roles are reviewed briefly in this section.

Cell Proliferation, Gene Silencing, and DNA Repair Biotin is hypothesized to exert effects on cell proliferation, gene silencing, and DNA repair through its attachment to histones in the nucleus of cells. Histones, of which there are four classes—H2A, H2B, H3, and H4—are small proteins that are bound to or associated with DNA as part of chromatin. Histones influence multiple processes. The attachment of biotin to histones is mediated by holocarboxylase synthetase and biotinidase. The biotinidase becomes biotinylated (i.e., forms biotinyl biotinidase) from the biotin moiety of biocytin and then transfers the biotin moiety to the histones at an alkaline pH [3,4,17,18]. The significance of the biotinylation is not clear, although effects on the cell cycle have been demonstrated. Biotinylation of histones appears to increase in response to cell proliferation. For example, mononucleated blood cells at the G1, S, G2, and M phases of the cell cycle displayed significantly more biotinylated histones versus quiescent cells [19,20]. In addition, biotinylation of histones has been found to affect gene slicing and cellular responses to some DNA damage [20–22].

Gene Expression and Cell Signalling Biotin (and possibly biotin catabolites) are also known to affect gene expression; in fact, more than 2000 human genes depend on biotin for expression [20]. Many (over 25%) of the genes that are biotin responsive play a role in cell signalling [20]. Effects of biotin on gene expression have been demonstrated in vitro and in animal studies. Specifically, biotin appears to be necessary for transcription of some genes and for translation of some mRNAs [21–25]. For example, biotin stimulates the expression (transcription) of glucokinase but inhibits the expression of phosphoenolpyruvate carboxykinase [18,23]. Biotin also increases mRNA levels of 6-phosphofructokinase when given to biotin-deficient rats, and mRNA levels of holocarboxylase synthetase are reduced with biotin deficiency and then increase in response to biotin supplementation [23]. Biotin's effects on gene expression are mediated by various cell signals and transcription factors such as biotinyl-AMP and cGMP, nuclear factors (NF)-κB, transcription factors Sp1 and Sp3, and receptor tyrosine kinases that span cell membranes [18,20,23]. The activity of these cell signals depends on biotin.

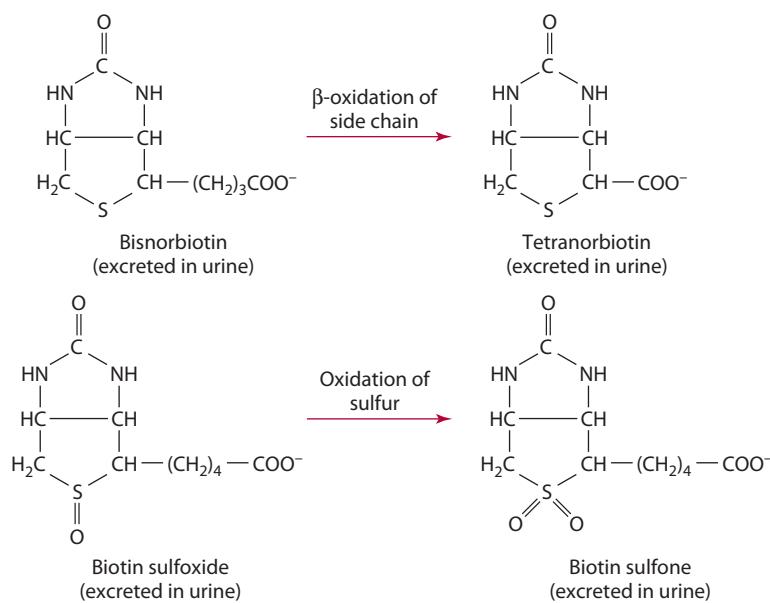


Figure 9.26 Selected metabolites from biotin degradation.

METABOLISM AND EXCRETION

Catabolism of the biotin holocarboxylases by proteases yields biotin oligopeptides and ultimately biocytin. The biocytin is then degraded by biotinidase to yield lysine and free biotin. Some of this biotin is excreted intact in the urine, and some may be reused or degraded [20].

In the catabolism of biotin, little degradation of the ring system of the vitamin occurs in humans. Most of the metabolites arise from degradation of the valeric acid side chain of biotin by β-oxidation. The main metabolites following catabolism of this side chain are bisnorbiotin and tetrnorbiotin (Figure 9.26) and, to a lesser degree, other derived metabolites such as bisnorbiotin methyl ketone and tetrnorbiotin methyl ketone [6,26]. Bisnorbiotin and tetrnorbiotin, along with the other metabolites, are excreted in the urine. Small amounts of other biotin metabolites are formed from oxidation of the sulfur in biotin's ring. These metabolites, which also are excreted in the urine, include biotin sulfoxide and biotin sulfone (Figure 9.26) [6,26,27]. Smoking appears to accelerate biotin catabolism in women [28].

Biotin that has been synthesized by intestinal bacteria but not absorbed is excreted in the feces. Very little dietary biotin that is absorbed is excreted in the feces [5].

ADEQUATE INTAKE

Because intestinally synthesized biotin is not sufficient to maintain normal biotin status, humans need to obtain biotin from the diet. An adequate intake (AI) recommendation for adults age 19 years and older of 30 µg biotin per day has been suggested [16]. As discussed earlier in this chapter under pantothenic acid, an AI is used instead of an RDA when insufficient data are available to

establish an estimated average requirement (EAR) and subsequent RDA [16]. Adequate intakes for biotin of 30 and 35 µg per day are suggested for women during pregnancy and lactation, respectively [16]. The inside covers of this book provide additional AIs for biotin for other age groups.

DEFICIENCY

Biotin deficiency in humans is characterized by lethargy, depression, hallucinations, muscle pain, paresthesia in extremities, anorexia, nausea, alopecia (hair loss), and scaly, red dermatitis. In addition, decreases in plasma biotin and in some biotin-dependent enzyme activities, and alterations in the urinary excretion of biotin and some of its metabolites occur [6,15,29]. A diet devoid of biotin can result in decreased plasma biotin and in reduced biotin excretion in about 2 to 4 weeks [30,31].

Biotin deficiency or poor biotin status, though fairly rare, occurs in various populations. People who ingest raw eggs in excess amounts are likely to develop biotin deficiency because of impaired biotin absorption. Impaired biotin absorption also may occur with gastrointestinal disorders such as inflammatory bowel disease and achlorhydria (lack of hydrochloric acid in gastric juices), in people on anticonvulsant drug therapy, or in chronic consumers of excessive amounts of alcohol. Biotin status has been shown to decline in some women during pregnancy [15]. Biotin deficiency is known to be teratogenic in animals [32]. People with genetic defects involving biotinidase and holocarboxylase synthetase activities develop biotin deficiency unless treated with pharmacologic doses of biotin. Biotin supplements in amounts of 5 to 10 mg daily are used to treat biotinidase deficiency, and doses of 40 to 100 mg biotin daily

are effective in people with holocarboxylase synthetase deficiency [2].

TOXICITY

Toxicity of biotin has not been reported, and thus no tolerable upper intake level has been established [16]. For example, fairly large doses (100 mg or more) of biotin have been given daily to people with inherited disorders of biotin metabolism without side effects [16]. Use of biotin as hair and skin conditioning agents in cosmetics has also been shown to be safe [33].

ASSESSMENT OF NUTRITUDE

Evaluation of biotin in blood as well as in urine has most often been used to assess biotin status. Low plasma concentrations of biotin, however, have not been shown to accurately reflect intake or status [16].

Decreased urinary biotin excretion (<6 µg/day or <200 pg/mL) coupled with increased urinary excretion of 3-hydroxyisovaleric acid generated from altered metabolism of β-methylcrotonyl CoA has been shown to be a sensitive indicator of biotin deficiency [15,16]. In fact, alterations in urinary 3-hydroxyisovaleric acid excretion, which can be detected within about 2 weeks of ingesting a biotin-deficient diet, are thought to be a more sensitive indicator than urinary biotin excretion [31,34].

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Folate

Folic acid is the term used to refer to the oxidized form of the vitamin found in fortified foods and in supplements. *Folate* refers to the reduced form of the vitamin found naturally in foods and in biological tissues. The Latin word *folium* means “leaf,” and the word *folate* from Italian means “foliage.”

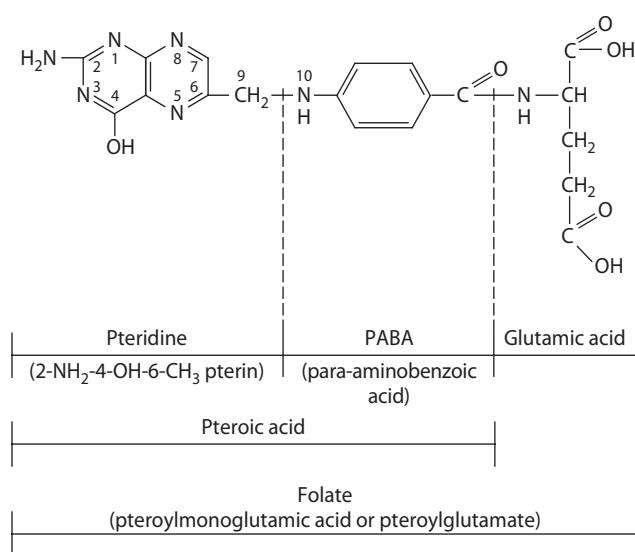


Figure 9.27 Structural formula of folate. Folate is made up of pteridine, which is conjugated by a methylene group ($-\text{CH}_2-$) to PABA forming pteroic acid. The carboxy group ($-\text{CO}-$) of PABA is peptide-bound to the amino group ($-\text{NH}-$) of glutamic acid to form folate.

Folate is made up of three distinct parts, all of which must be present for vitamin activity. Figure 9.27 shows the structure of folate. As shown in the figure, 2-amino-4-hydroxypteridine, also called pteridine or pterin, is conjugated by a methylene group ($-\text{CH}_2-$) to paraaminobenzoic acid (PABA) to form pteroic acid. The carboxy group of PABA is peptide-bound to the amino group of glutamic acid to form folate, which is also called pteroylglutamate or pteroylmonoglutamate [1]. In the body, metabolically active folate has multiple glutamic acid residues attached. Although humans can synthesize all the component parts of the vitamin, they do not have the enzyme necessary for the coupling of the pterin molecule to PABA to form pteroic acid [1].

Folate's and vitamin B₁₂'s discovery resulted from the search to cure the disorder megaloblastic anemia, a problem in the late 1870s and early 1880s. As with many of the other vitamins, eating liver was shown to cure the condition. Mitchell and associates are credited with the vitamin's discovery in 1941.

SOURCES

Good food sources of folate include mushrooms and green vegetables such as spinach, brussels sprouts, broccoli, asparagus, and turnip greens, okra, among others, as well as peanuts, legumes (especially lima, pinto, and kidney beans), lentils, fruits (especially strawberries and oranges) and their juices, and liver. Raw foods typically are higher in folate than cooked foods because of folate losses incurred with cooking. Fortification of flours, grains, and cereals with folic acid (140 µg folic acid per 100 g of product) was

initiated in 1998. Thus, fortified cereals, breads, and grain products now represent major sources of the vitamin. Some juices also are now fortified with folic acid.

Folate in foods exists primarily in the reduced form and it usually contains up to nine glutamic acid residues instead of the one glutamate shown later in Figure 9.30. The principal pteroylpolyglutamates in foods are 5-methyl tetrahydrofolate (THF) and 10-formyl THF, although over 150 different forms of folate have been reported [2,3]. In supplements and in fortified foods, folic acid is provided as pteroylmonoglutamate, the most oxidized and stable form of the vitamin. As a supplement, folic acid is almost completely available (especially if consumed on an empty stomach). When fortified foods are consumed with natural sources of folate, the vitamin is about 85% bioavailable.

Folate bioavailability from foods varies, from about 10% to 98%, because of a variety of factors [4]. Variations in intestinal conditions such as pH, genetic variations in enzymatic activity needed for folate digestion, dietary constituents such as inhibitors, and the food matrix, for example, influence bioavailability. Reduced forms of folate pteroylpolyglutamates in foods are labile and easily oxidized. The folate in milk is bound to a high-affinity folate-binding-protein, which appears to enhance its bioavailability. Generally, folate bioavailability from a mixed diet is thought to be about 50% [5]. Because of the difference in the efficiency of folate absorption from foods versus folic acid from supplements and fortified grain products, folate equivalents are used in recommendations for dietary folate intakes (see Recommended Dietary Allowances in this section) [1,6,7].

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Before the polyglutamate forms of folate in foods can be absorbed, they must be hydrolyzed to the monoglutamate form. This hydrolysis or deconjugation is performed by at least two folylpoly γ -glutamyl carboxypeptidases (FGCP), also called pteroylpolyglutamate hydrolases or conjugases. The conjugases exhibit separate activities in the human jejunal mucosa, one soluble and the other membrane bound in the intestinal brush border [2]. The conjugases are also found in the pancreatic juice and bile. The brush border conjugase is a zinc-dependent exopeptidase that stepwise cleaves the polyglutamate into mono-glutamate. Zinc deficiency impairs conjugase activity and diminishes digestion and thus absorption of folate [6]. Chronic alcohol ingestion and conjugase inhibitors in foods such as legumes, lentils, cabbage, and oranges also diminish conjugase activity to impair digestion of polyglutamate forms of folate and thus inhibit folate absorption. Folic acid in fortified foods does not need to undergo digestion because it already is present in the monoglutamate form.

Enzymes involved in interconversions of coenzyme forms of THF:

- ① Serine hydroxymethyltransferase (coenzyme-PLP)
- ② Methylene THF dehydrogenase
- ③ Cyclohydrolase
- ④ Formate-activating enzyme
- ⑤ Methylene-THF reductase
- ⑥ Methionine synthetase (coenzyme-B₁₂)
- ⑦ Formiminotransferase
- ⑧ Cyclodeaminase
- ⑨ Thymidylate synthetase
- ⑩ 10-formyl THF dehydrogenase

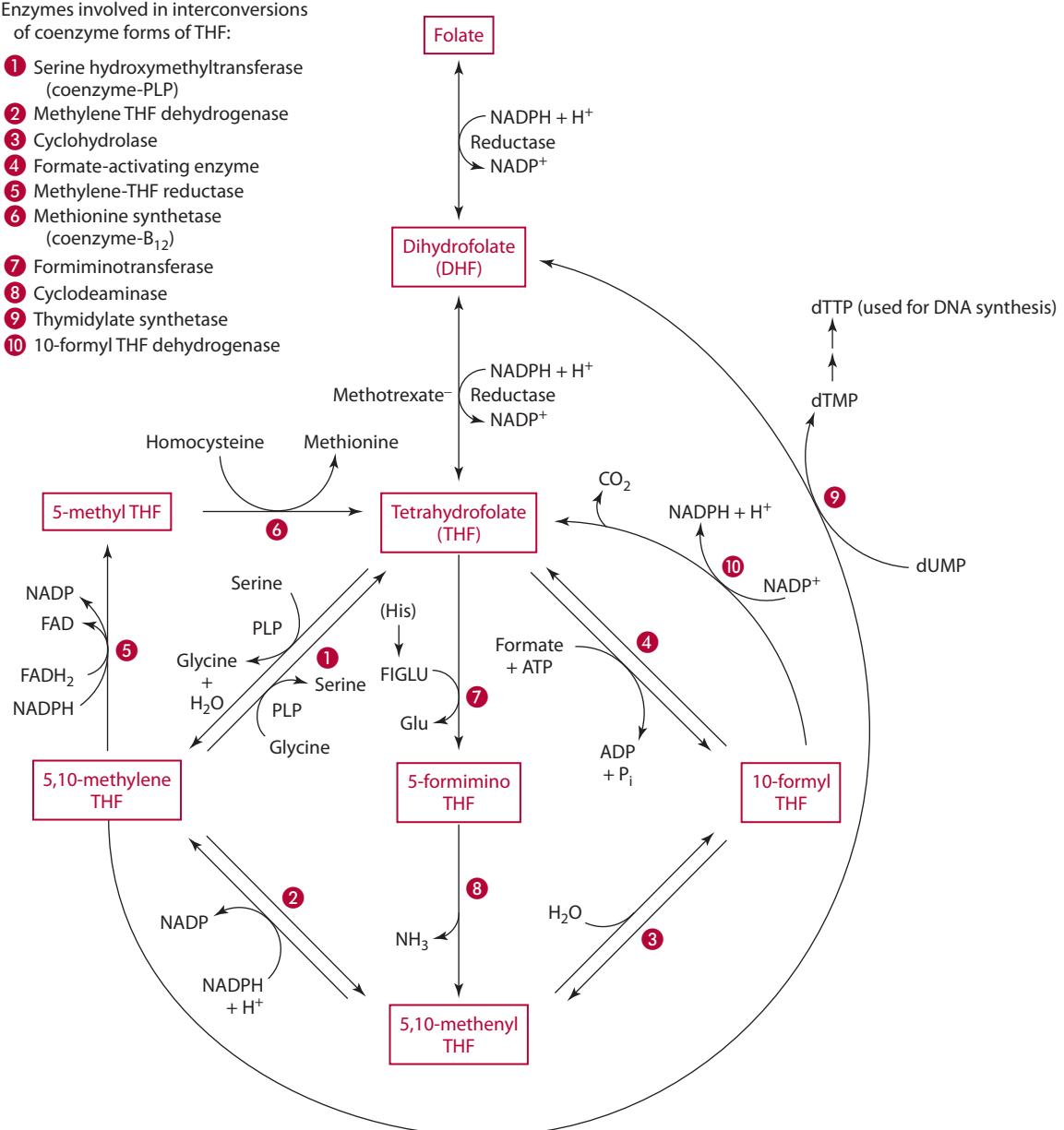


Figure 9.28 Interconversions of coenzyme forms of tetrahydrofolate (THF).

Several carriers appear to transport folate into intestinal cells. These folate carriers are also sometimes called folate-binding proteins (FBPs) or folate receptors. One carrier system in the proximal small intestine that transports folate (in its reduced form) is saturable and dependent on pH, energy, and sodium [2,3,8]. Another folate protein carrier called the reduced folate transporter or carrier (RFT or RFC or FOLT), found in white blood cells but also appears to be in many other tissues (including the intestine) in the body, transports 5-methyl THF into cells [9]. Diffusion also accounts for some folate absorption, especially when pharmacological doses of the vitamin are consumed. Absorption occurs throughout the small intestine but is most efficient in the jejunum.

Within the intestinal cell, folate and subsequently dihydrofolate (DHF) are reduced to generate THF (Figure 9.28). The reduction of various folate forms to THF occurs stepwise through the action of NADPH-dependent dihydrofolate reductase, a cytoplasmic enzyme (Figure 9.28). Four additional hydrogens are added at positions 5, 6, 7, and 8. THF is then either methylated in a two-step reaction to 5-methyl THF or formylated (Figure 9.28). Folate-binding proteins are thought to transport folate within cells. Folate is found in portal circulation as 5-methyl THF, although dihydrofolate and formylated forms are also present; all forms are as mono- and not polyglutamates. Transport of folate across the basolateral membrane to enter the blood is carrier dependent.

Uptake of folate into the liver (and other tissues) is carrier mediated by folate receptors, although diffusion is also responsible for the uptake of folate into some tissues [8–10]. Some of the folate receptors include RFT (also found in the intestine); folate receptor α , which transports 5-methyl THF monoglutamate; and folate receptor β , which has not been fully characterized [9]. Within liver cells, dihydrofolate is typically reduced to THF and conjugated to glutamates either for storage or for conversion to 5-methyl THF [2]. Within the liver, about 33% of folate is present as THF, 37% as 5-methyl THF, 23% as 10-formyl THF, and 7% as 5-formyl THF [11]. Bound to the various folates, especially to 5-methyl THF and 10-formyl THF, are glutamates typically varying in length from 3 to 9. Folylylpolyglutamate synthetase, found in a variety of body tissues, catalyzes the ATP-dependent additions of the glutamates. Glutamate residues are usually added one at a time to the monoglutamate. Addition of these glutamate residues not only allows the production of the various folate coenzyme forms but also traps the folate in the cell. Moreover, the polyglutamate form of folate is a better substrate for folate-dependent enzymes than the monoglutamate form. Before release into systemic blood, however, glutamate residues are removed from folate polyglutamates by hydrolases.

In the blood, folate is found as a monoglutamate. Almost two-thirds of the folate in systemic blood plasma is bound to proteins; free folate accounts for the other one-third [12]. In the blood, folate-binding proteins bind folate with high affinity; albumin and α -2 macroglobulin also bind folate, but with relatively low affinity. Monoglutamate forms of folate in the blood include THF, 5-methyl THF, and 10-formyl THF, among other forms [2]. Red blood cells contain more folate than does plasma; however, red blood cell folate is attained during erythropoiesis. In other words, folate is not taken up by mature red blood cells. Thus, red blood cell folate concentrations represent an index of longer-term (2 to 3 months) folate status than does plasma.

Total body folate levels range from about 11 to 28 mg [1]. The liver stores about one-half of the body's folate. The main storage forms are the polyglutamate forms of THF and 5-methyl THF. Storage may occur in association with intracellular folate-binding proteins [12].

Folate is found in both the cytoplasm and mitochondria of cells, where it functions as a coenzyme (or cosubstrate) to accept and donate one-carbon units. Availability of folate to crucial tissues where rapid cell division is occurring appears to be carefully regulated when the supply of dietary folate is limited. The mechanisms of regulation are unclear, but regulation seems to occur through changes in the rate of synthesis of polyglutamates. The less metabolically active tissues return folate monoglutamates to the liver, which then redistributes the folate to the actively proliferating cells. How circulating folates are directed to specific tissues is uncertain, but folate-binding proteins (folate receptors) may provide tissue-specific uptake [11,12].

FUNCTIONS AND MECHANISMS OF ACTION

THF functions in the body as a coenzyme in both the mitochondria and cytoplasm to accept one-carbon groups typically generated from amino acid metabolism. These THF derivatives then serve as donors of one-carbon units in a variety of synthetic reactions, such as dispensable amino acid synthesis and purine and pyrimidine synthesis. The one-carbon group accepted by THF is bonded to its nitrogen in position 5 or 10 or to both (Figure 9.28). The coenzyme forms are interconvertible, except that 5-methyl THF cannot be converted back to 5,10-methylene THF (Figure 9.28). Genetic polymorphisms in some of the folate-dependent enzymes have been identified. Several mutations in methylene THF reductase (sometimes abbreviated MTHFR) have been demonstrated. MTHFR converts 5,10-methylene THF to 5-methyl THF. The enzyme requires riboflavin as FAD as a prosthetic group. Mutations in MTHFR impair 5-methyl THF formation and thus reduce remethylation of homocysteine, resulting in hyperhomocysteinemia, a risk factor for heart disease.

The THF derivatives, which participate in a variety of reactions, are illustrated as follows:

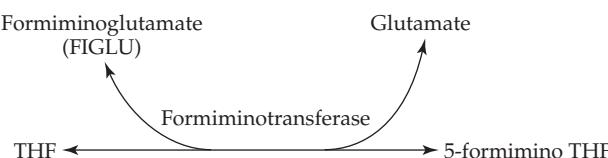
5- and 10-formyl THF	$\text{O}=\text{CH}$
5-formimino THF	$-\text{HC}=\text{NH}-$
5,10-methenyl THF	$=\text{CH}-$
5,10-methylene THF	$-\text{CH}_2-$
5-methyl THF	$-\text{CH}_3$

The formyl derivatives represent the most oxidized forms of folate, and 5-methyl THF is the most reduced form.

Amino Acid Metabolism

Folate is involved in the metabolism of several amino acids, including histidine, serine, glycine, and methionine.

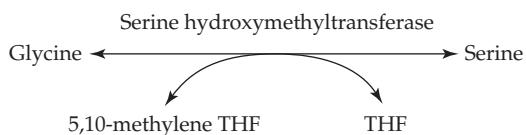
Histidine Histidine metabolism requires THF. Deamination of histidine generates urocanic acid, which can undergo further metabolism to yield formiminoglutamate (FIGLU). The formimino group is removed from FIGLU with the help of formiminotransferase to generate glutamate. THF receives the formimino group to yield 5-formimino THF, as shown below and in Figure 9.29.



This reaction can be used as a basis for determining folate deficiency. In the medical procedure, subjects are given an oral histidine load, and FIGLU excretion is measured in the urine. With folate deficiency, FIGLU accumulates

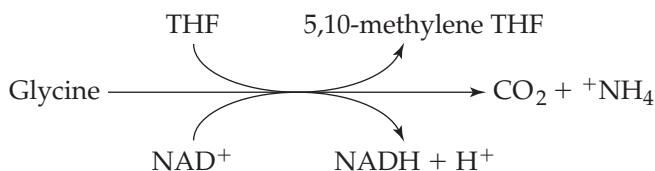
in the blood and is excreted in higher concentrations in the urine, because if THF were available FIGLU would be converted into glutamate.

Serine and Glycine Folate as 5,10-methylene THF is required for serine synthesis from glycine (Figure 9.28). The 5,10-methylene THF contributes a hydroxymethyl group to glycine to produce serine. Serine represents a major source of one-carbon units for use in folate reactions. Vitamin B₆ as pyridoxal phosphate (PLP) is required for serine hydroxymethyltransferase activity. This enzyme is found in all tissues, especially the liver and kidney, in both the cytoplasm and mitochondria.



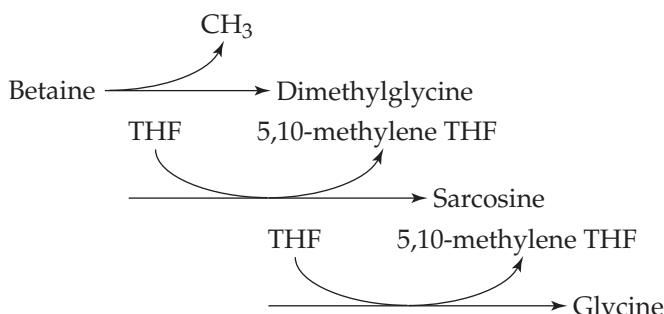
The conversion of glycine to serine is reversible such that glycine may be synthesized from serine in a THF-dependent reaction. However, the direction of the reaction varies between tissues; some tissues, such as the kidney, generate more serine, whereas others generate more glycine.

Some other reactions involving glycine metabolism also require folate. Glycine degradation, for example, requires THF as shown below and later in Figure 6.32.



Glycine synthesis from choline degradation also involves folate. Choline is catabolized in the liver in an NAD⁺-dependent reaction to form betaine and NADH + H⁺ [13]. Betaine, also called trimethyl glycine, functions as a methyl donor that provides methyl groups to other compounds, such as homocysteine, through a methyltransferase, generating dimethylglycine. The dimethylglycine

resulting from demethylation of betaine undergoes further metabolism to generate sarcosine in a reaction catalyzed by dimethylglycine dehydrogenase. In that reaction and the subsequent reaction catalyzed by sarcosine dehydrogenase (in which sarcosine, also called monomethylglycine, is converted to glycine), THF functions as the carbon acceptor, forming 5,10-methylene THF. Riboflavin as FAD also is required. Betaine oxidation occurs primarily in the mitochondria of liver and kidney cells [13].



Betaine is found both in animal and plant foods. Estimated betaine intake ranges from about 1 to 2.5 g per day. Folic acid supplementation appears to increase betaine concentrations [14], and betaine appears to be able to reduce plasma homocysteine concentrations in those with elevated blood levels [13].

Methionine Methionine regeneration from homocysteine also involves folate as 5-methyl THF. Before addressing this regeneration, consider this brief overview of the conversion of methionine to homocysteine. As shown in Figure 9.30, methionine is converted to S-adenosyl methionine (SAM) in an ATP-requiring reaction catalyzed by methionine adenosyl transferase. The removal of the methyl group from SAM results in the formation of S-adenosyl homocysteine (SAH). Removal of the adenosyl group from SAH yields homocysteine.

Folate is needed for the remethylation of homocysteine to form methionine. This reaction, which occurs when

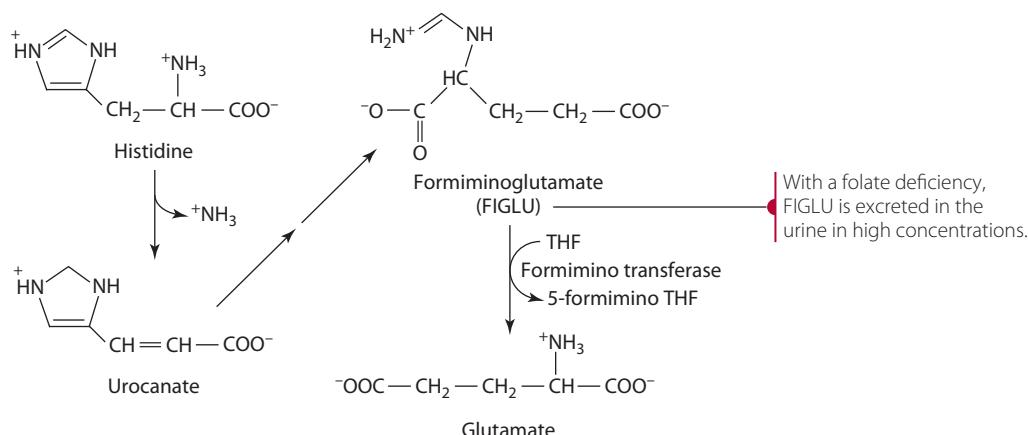
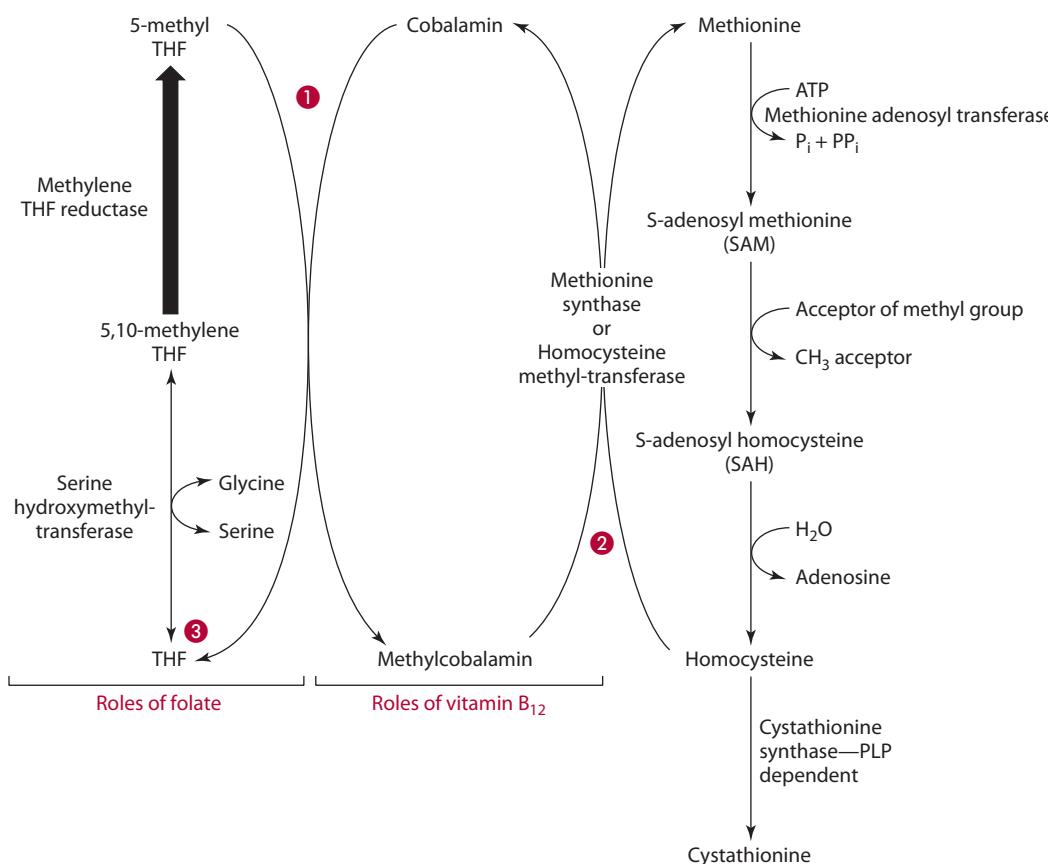


Figure 9.29 The role of folate in histidine catabolism.



- ① Cobalamin, which is bound to the enzyme methionine synthase, picks up the methyl group on 5-methyl THF forming THF and methylcobalamin.
- ② Methylcobalamin, which is still bound to the enzyme methionine synthase, gives the methyl group to homocysteine which then forms methionine and reforms cobalamin.
- ③ THF must be reconverted to 5-methyl THF for the reaction to proceed again. This process requires two reactions catalyzed first by serine hydroxymethyl transferase to generate 5,10 methylene THF. Second, methylene THF reductase converts 5,10 methylene THF to 5-methyl THF, which can once again donate its methyl group to cobalamin.

Figure 9.30 The resynthesis of methionine from homocysteine, showing the roles of folate and vitamin B₁₂.

SAM concentrations are low, requires folate as 5-methyl THF as a methyl donor and vitamin B₁₂ in the form of methylcobalamin as a prosthetic group for homocysteine methyltransferase (also called methionine synthase). For methionine synthase to transfer a methyl group from 5-methyl THF to homocysteine, cobalamin must be tightly bound to the enzyme. While bound to methionine synthase, cobalamin picks up the methyl group from 5-methyl THF to generate methylcobalamin and THF. Methylcobalamin then serves as the methyl donor for converting homocysteine to methionine. These reactions are shown in Figure 9.30 and are broken down into steps to facilitate understanding in the “Functions and Mechanisms of Action” section under vitamin B₁₂.

SAM is an important compound, serving as a methyl donor in many reactions in the body. For example, DNA and RNA methylation, myelin maintenance, neural function, and polyamine, carnitine, and catecholamine synthesis, among other processes, are dependent on methylation reactions using SAM. SAM concentrations in part regulate methionine metabolism, including the

remethylation of homocysteine to methionine. SAM concentrations increase with increased methionine. Higher SAM concentrations stimulate the transsulfuration pathway in which homocysteine is converted, in an irreversible reaction, to cystathione by vitamin B₆-dependent cystathione synthase (Figure 9.30) and ultimately is converted to cysteine. Higher SAM concentrations inhibit methylene THF reductase, which converts 5,10-methylene THF to 5-methyl THF. This inhibition decreases 5-methyl THF availability to decrease remethylation of homocysteine.

Possible Relationships with Diseases The roles of folate and vitamin B₁₂ in the conversion of homocysteine to methionine, along with the role of vitamin B₆ in the conversion of homocysteine to cystathione (Figure 9.30), continue to receive considerable attention because low intakes of these three vitamins, especially folate, are inversely associated with plasma homocysteine concentrations, and elevated plasma homocysteine concentrations (>15 μ) are associated with premature coronary artery disease, as well

as with premature occlusive vascular disease and cerebral or peripheral vascular disease [15–19].

The mechanisms by which hyperhomocysteinemia increases vascular disease risk have not been elucidated, but it may act by impairing endothelial function, promoting the growth of smooth muscle cells leading to vascular lesions, or promoting platelet adhesiveness and clotting, among other hypotheses [20–22]. Research suggests that an increase of 5 µM/L in plasma homocysteine concentrations increases the risk for heart disease as much as an increase of 0.5 mmol/L (20 mg/dL) in plasma cholesterol concentrations [16]. Supplementation of folic acid, vitamin B₁₂, and vitamin B₆ in people (both healthy and with heart disease) with hyperhomocysteinemia (high blood homocysteine concentrations) normalizes or reduces blood homocysteine concentrations and thus decreases heart disease risk [23,24]. Folic acid supplementation in people with hyperhomocysteinemia also improves endothelial function, and moderate to high folate concentrations in the blood have been associated with a reduced incidence of coronary events [17,25]. However, whether folate has direct effects on the development of heart disease has not been determined [26].

Another condition being investigated as possibly linked to poor folate status is dementia, including Alzheimer's dementia [27]. Memory and abstract thinking appear to be influenced by folate. Cognitive dysfunction and dementia have been shown to correlate with plasma homocysteine concentrations, which in turn are influenced in part by folate status [28,29].

Purine and Pyrimidine Synthesis/Nucleotide Metabolism

The involvement of THF derivatives in purine and pyrimidine synthesis (Figures 6.16–6.20) makes folate essential for cell division. Synthesis of cells with short life spans, such as enterocytes, is particularly dependent on adequate levels of folate. In pyrimidine synthesis, thymidylate synthetase uses 5,10-methylene THF to convert dUMP to dTMP and dihydrofolate (DHF) (Figure 9.28). dTMP is required for DNA synthesis. To regenerate 5,10-methylene THF, DHF is converted by dihydroreductase to THF in a reaction requiring NADPH. The THF is converted to 5,10-methylene THF as serine is converted to glycine by serine hydroxymethyl transferase. Both thymidylate synthetase and dihydrofolate reductase are active enzymes in cells undergoing cell division. Inhibitors of dihydrofolate reductase, such as the therapeutic drug methotrexate, which binds to the enzyme's active site, have been employed in treating cancer, rheumatoid arthritis, and psoriasis, among other conditions, to prevent synthesis of THF needed for actively dividing cells.

Folate as 10-formyl THF is needed for purine (adenine and guanine) ring formation. In the purine ring, carbon

atom 8 involves the formylation of 5-phosphoribosylglycinamide or called glycinamide ribotide (GAR) to form 5-phosphoribosyl formylglycinamide ribotide (FGAR). 10-formyl THF donates the formyl group in this reaction (Figures 6.18 and 6.19). Purine-ring carbon atom 2 is acquired by formylation of 5-phosphoribosyl 5-amino 4-imidazole 4-carboxamide ribonucleotide (AICAR). 10-formyl THF formylates AICAR to generate 5-phosphoribosyl 5-formamido 4-imidazole carboxamide ribotide (FAICAR) (Figures 6.18 and 6.19).

Other Relationships with Diseases Folate deficiency or poor folate status is also suspected in the development (initiation) of some cancers, especially colon cancer. Folate deficiency in cells and tissues is thought to increase the potential for neoplastic changes in normal cells during the early stages of cancer [30,31]. Some evidence suggests that decreased methylation of DNA, especially tumor suppression genes, or increased DNA strand breaks (associated with misincorporation of uridylate for thymidylate in DNA, caused by folate deficiency) may alter gene expression and thus promote cancer [32–34]. Lower folate intakes have been associated with some cancers, and conversely higher folate intakes have been associated with reduced risks for some cancers [35–37]. Polymorphisms in methylenetetrahydrofolate reductase can increase the likelihood for colorectal cancers, as discussed in more detail in the Perspective at the end of this chapter.

INTERACTIONS WITH OTHER NUTRIENTS

A synergistic relationship exists between folate and vitamin B₁₂, also called cobalamin. This relationship, whereby without vitamin B₁₂ the methyl group from 5-methyl THF can't be removed and thus is trapped, is sometimes called the "methyl-folate trap." The following sequence of events leads to the methyl-folate trap (tracing the reactions shown in Figures 9.28 and 9.30 is helpful). Serine donates single carbons through conversion to glycine, and in the process THF is converted to 5,10-methylene THF. The 5,10-methylene THF is readily reduced to 5-methyl THF by a reductase (whose activity is inhibited by its end product, 5-methyl THF, and by SAM). 5-methyl THF is required for methionine synthesis from homocysteine. Methyl groups are transferred by the enzyme methionine synthase from 5-methyl THF to vitamin B₁₂. Adequate vitamin B₁₂ must be present for the activity of methionine synthase. The addition of the methyl group to cobalamin generates methylcobalamin, which serves as the methyl donor for converting homocysteine to methionine. Without cobalamin to accept the methyl group from 5-methyl THF, the 5-methyl THF accumulates and is trapped, and THF is not regenerated. With adequate vitamin B₁₂ status, the THF resulting from the synthesis of methionine is needed as a

substrate for conversion into its various other coenzyme forms. For example, 10-formyl THF is needed for purine synthesis, and 5,10-methylene THF is needed for thymidylate synthesis, which in turn must be present for DNA synthesis. Thus, the synergism between folate and vitamin B₁₂ is very important for support of rapidly proliferating cells. See “Toxicity” in this section for a discussion on the ability of folate supplements to mask vitamin B₁₂ deficiency.

METABOLISM AND EXCRETION

Folate is excreted from the body in both the urine and the feces. Within the kidney, folate-binding proteins present in the renal brush border and coupled with tubular reabsorption of folate help the body retain needed folate. Excess folate is excreted in the urine with some folate excreted intact and some catabolized in the liver prior to excretion. Oxidative cleavage of folate is thought to occur between C9 and N10 of polyglutamate forms of the vitamin. This cleavage generates para-aminobenzoyl polyglutamate. Glutamate residues (except for one) are then hydrolyzed, and usually the compound is acetylated to form the major urinary metabolite N-acetyl paraaminobenzoyl glutamate. Smaller amounts of para-aminobenzoyl glutamate also are found in the urine.

In addition to urinary losses, folate (up to about 100 µg) is secreted by the liver into the bile. Most of this folate, however, is reabsorbed following enterohepatic recirculation, so losses in the stool are minimal [1,38]. Folate of microbial origin, however, may appear in the feces in relatively high amounts.

RECOMMENDED DIETARY ALLOWANCE

The 1998 RDA for folate considers its bioavailability as well as several indices of nutriture in its determination. The RDA for adults for folate is 400 µg dietary folate equivalents (DFE) per day [1]. Folate requirements are estimated at 320 µg per day [1]. The Centers for Disease Control and Prevention suggest 400 µg synthetic folic acid/day for women capable of becoming pregnant because of evidence that folic acid supplementation during the periconceptional period of pregnancy may reduce the incidence of neural tube defects [39,40]. Foods that are good sources of folate (that is, the food provides either $\geq 10\%$ of the Daily Value or at least 40 µg/serving) are permitted by the Food and Drug Administration to make the health claim “Healthful diets with adequate folate may reduce a woman’s risk of having a child with a neural tube (brain or spinal cord) defect” [40]. RDAs for folate of 600 µg and 500 µg of DFE per day are suggested for pregnancy and lactation, respectively [1]. One DFE is equal to 1 µg of food folate, which is equal to 0.6 µg of folic acid from a supplement or fortified food consumed with a meal, which is equal to 0.5 µg of

folic acid from a supplement taken without food (empty stomach) [1]. Stated alternately, the DFE is the quantity of folate in natural foods plus 1.7 times the amount of folic acid in the diet; the definition is based on the assumption that the bioavailability of folic acid supplemented in foods is greater than folate found naturally in foods by a factor of 1.7 or 2 times the amount of synthetic folic acid when ingested with an empty stomach [4]. Additional RDAs for folate for other age groups are provided on the inside covers of the book.

DEFICIENCY: MEGLOBLASTIC MACROCYTIC ANEMIA

Marginal folate deficiency results in megaloblastic macrocytic anemia. The deficiency is characterized initially (within a month if the diet is devoid of folate) by low plasma folate. Red blood cell folate concentrations diminish after about 3 to 4 months of low folate intake [30]. After approximately 4 to 5 months, bone marrow cells and other rapidly dividing cells become megaloblastic [30]. Mean cell volume (MCV) increases, and hypersegmentation (increased lobes) of white blood cells (neutrophils) occurs, along with decreased blood cell counts. People with folate deficiency may exhibit fatigue, weakness, headaches, irritability, difficulty concentrating, shortness of breath, and palpitations, among other symptoms.

Megaloblastic anemia—the release into circulation of red blood cells that are fewer than normal and large and immature—related to folate deficiency is relatively common in the United States. However, megaloblastic anemia also occurs with deficiency of vitamin B₁₂. The anemia results from abnormal DNA synthesis and failure of blood cells to divide properly, coupled with the continued formation of RNA. The quantity of RNA becomes greater than normal, leading to excess production of other cytoplasmic constituents, including hemoglobin. The result is immature, enlarged macrocytic cells often containing excessive hemoglobin.

Figure 9.31 reviews the formation and maturation of erythrocytes and may better illustrate the effects of a folate and vitamin B₁₂ deficiency. Briefly, the proerythroblast develops from stem cells in bone marrow under the stimulation of hypoxia (low blood oxygen) in the presence of erythropoietin (a hormone produced in the kidney). In the proerythroblast, active DNA and RNA synthesis occurs and cell division begins. Cells resulting from the first division are given the name basophilic erythroblasts because they stain blue with basic dyes because of the many organelles present within the cell. During this stage, hemoglobin synthesis begins. The next generation of cells consists of the polychromatophil erythroblasts, in which hemoglobin synthesis intensifies. The concentration of hemoglobin influences DNA synthesis and cell division.

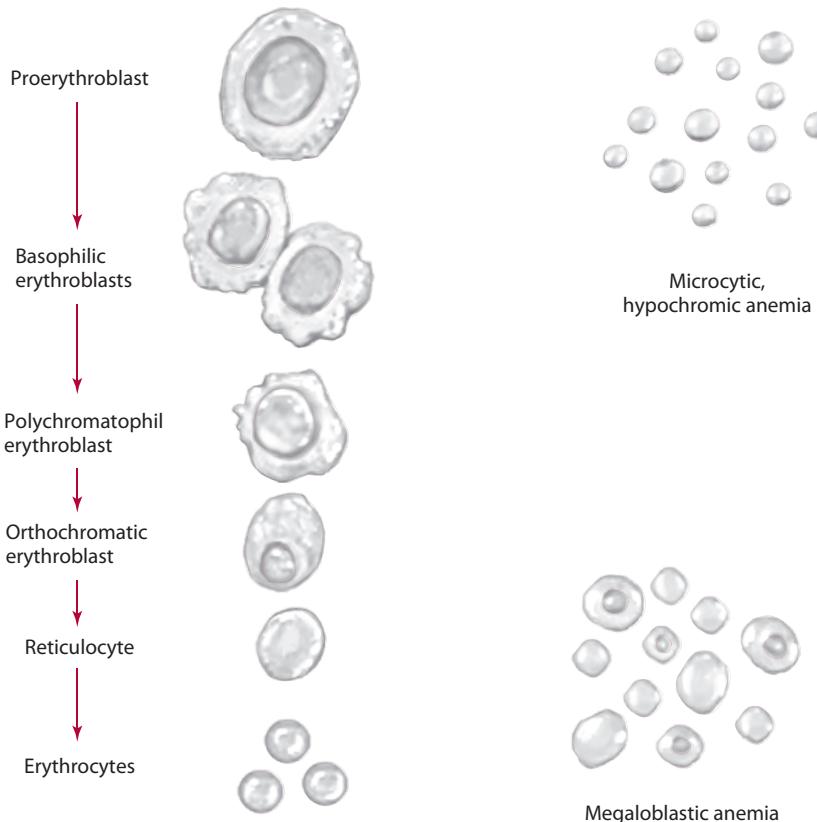
Genesis of RBC

Figure 9.31 Genesis and maturation of the red blood cells (left); red blood cells characteristic of microcytic and megaloblastic anemias (right).

Cell division usually continues into the orthochromatic stage. The orthochromatic erythroblasts are characterized by continued hemoglobin synthesis, discontinuation of DNA synthesis, a slowing of RNA synthesis, and migration of the nucleus to the cell wall in preparation for extrusion. The cell now becomes the reticulocyte, in which hemoglobin synthesis continues up to a concentration of approximately 34%. Once this concentration is reached, the ribosomes disappear and the cells pass into blood capillaries by squeezing through pores of the membrane. In about 2 to 3 days, when the rest of the cell organelles have disappeared, the reticulocytes become erythrocytes. The erythrocyte, or mature red blood cell, is all cytoplasm packed with hemoglobin. Glycolysis and hexose monophosphate shunt are the only metabolic pathways occurring in the erythrocyte.

A deficiency of folate or vitamin B₁₂ interferes with normal cell division. With folate deficiency, increased strand breaks occur within the DNA, and imbalances in DNA bases occur with decreased conversion of deoxyuridylate to thymidylate, which is required for DNA synthesis and thus normal cell division. Problems are theorized to arise in part because uracil may be misincorporated for thymidylate into DNA. Although enzymes can remove the uracil, lack of thymidine triphosphate (TTP) inhibits repair of the DNA [32–34]. Without appropriate repair, double strand breaks and fragmentation occur in

the DNA [34,41,42]. Over time, cell division diminishes. Large, malformed, and sometimes nucleated red blood cells result.

Some conditions and populations associated with increased need for folate intake include people with excessive alcohol ingestion, those with malabsorption disorders such as inflammatory bowel diseases, and people taking certain medications. Folate deficiency has been observed in people taking diphenylhydantoin or phenytoin, anticonvulsants used to treat epilepsy. Folate and phenytoin each inhibit the cellular uptake of the other in the gastrointestinal tract and possibly in the brain [1]. Methotrexate, used to treat rheumatoid arthritis and some cancers, among other conditions, binds to dihydrofolate reductase and thus prevents THF synthesis (Figure 9.28). Other drugs, including cholestyramine (used to treat high cholesterol concentrations) and sulfasalazine (used to treat inflammatory bowel diseases), have also been shown to interact with folate to create folate deficiency. Conditions such as inflammatory bowel diseases (e.g., Crohn's and ulcerative colitis) and excessive alcohol ingestion are known to cause malabsorption of folate. Other populations that appear to have an increased need for folate include those with the genetic polymorphisms in enzymes involved in folate metabolism such as the MTHFR 677C → T variant (see the Perspective at the end of this chapter).

TOXICITY

Toxicity of oral folic acid in moderate doses reportedly is virtually nonexistent [1,43]. Other studies, however, indicate that folate intakes of up to 15 mg daily are problematic. Problems include insomnia, malaise, irritability, and gastrointestinal distress [44]. Folic acid supplementation also is problematic if it masks a vitamin B₁₂ deficiency [45,46]. Folic acid supplements can alleviate the megaloblastic anemia caused by a vitamin B₁₂ deficiency, but the neurological damage caused by the deficiency progresses undetected. A tolerable upper intake level for adults of 1,000 µg (1 mg) for synthetic folic acid in supplements or from fortified foods (not natural foods) has been suggested based on the ability of folate to mask the neurological manifestations of vitamin B₁₂ deficiency [1]. Use of folic acid supplements is usually discouraged for some people, such as those with cancer receiving chemotherapy with methotrexate.

ASSESSMENT OF NUTRITUDE

Folate status is most often assessed by measuring folate concentrations in the plasma, serum, or red blood cells. Serum or plasma folate levels reflect recent dietary intake; thus, true deficiency must be interpreted through repeated measures of serum or plasma folate. Serum folate concentrations <6.8 ng/mL typically suggest deficiency [1,47]. Red blood cell folate concentrations are more reflective of folate tissue status than is serum folate and represent vitamin status at the time the red blood cells were synthesized [1,47]. Red blood cell folate concentrations <363 nmol/L suggest folate deficiency; however, concentrations are also lowered with a vitamin B₁₂ deficiency [1,47].

Formiminoglutamate (FIGLU) excretion may also be used to measure folate nutriture, because folate as THF must be available for the formimino group to be removed from FIGLU and glutamate to be formed (Figure 9.29). FIGLU excretion is measured in a 6-hour urine collection after ingestion of 2 to 5 g oral L-histidine. Normal FIGLU excretion is <35 µM /day in folate adequate adults, whereas with folate deficiency it rises to >200 µM /day [47]. A deficiency of vitamin B₁₂, however, also elevates FIGLU excretion.

The deoxyuridine suppression test, another method for assessing folate status, measures the availability of folate for de novo thymidine synthesis. In this test, the activity of thymidylate synthetase is measured in cultured lymphocytes or bone marrow cells. The reaction catalyzed by thymidylate synthetase is dependent on folate and, indirectly, on vitamin B₁₂; therefore, the change in activity elicited by adding one or the other vitamin allows the deficiency to be identified. In other words, if a person were folate deficient, adding folate—but not vitamin B₁₂—would normalize enzyme activity. Likewise, if a person were vitamin B₁₂ deficient, adding vitamin B₁₂—and not folate—would normalize thymidylate synthetase activity.

In the case of a deficiency of both vitamins, enzyme activity could be normalized only by adding both vitamins [47].

A functional marker of folate and vitamin B₁₂ deficiencies is elevated plasma homocysteine concentrations. Remember, both vitamins are required for the remethylation of homocysteine to methionine. With deficiency of either vitamin, plasma homocysteine concentrations become elevated.

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Vitamin B₁₂ (Cobalamin)

Vitamin B₁₂, also called cobalamin, is considered a generic term for a group of compounds called corrinoids because of their corrin nucleus. The corrin is a macrocyclic ring made of four reduced pyrrole rings linked together. In the center of the corrin is an atom of cobalt (Co) to which is attached, at almost right angles, the nucleotide 5,6-dimethylbenzimidazole. Also attached to the cobalt atom in vitamin B₁₂ is one of the following:

Group Attached	Resulting Compound
—CN	Cyanocobalamin
—OH	Hydroxocobalamin
—H ₂ O	Aquocobalamin
—NO ₂	Nitritocobalamin
5'-deoxyadenosyl	5'-deoxyadenosylcobalamin
—CH ₃	Methylcobalamin

The structure of cyanocobalamin is shown in Figure 9.32. Only two cobalamins, 5'-deoxyadenosylcobalamin (subsequently called adenosylcobalamin) and methylcobalamin, are active as coenzymes. The human

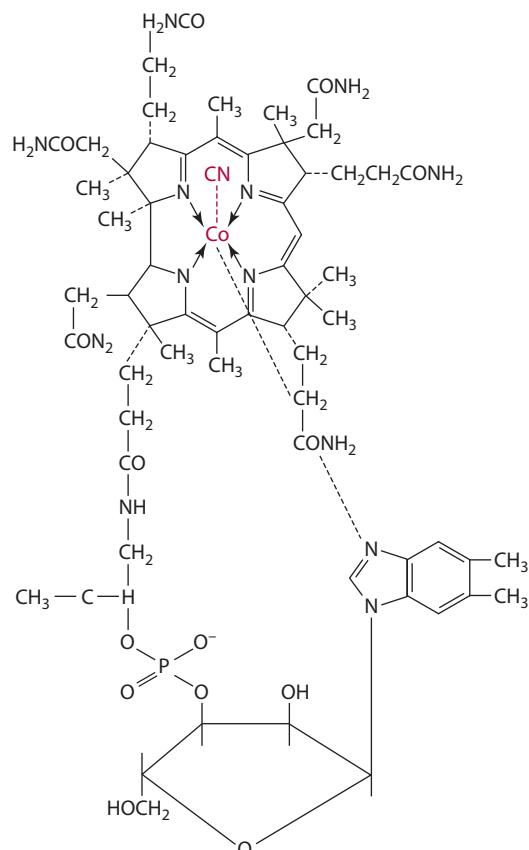


Figure 9.32 Structural formula of vitamin B₁₂ (cyanocobalamin).

body has the biochemical ability to convert most of the other cobalamins into an active coenzyme form of the vitamin.

Vitamin B₁₂ was the last vitamin to be discovered. It was isolated in 1948 by Smith (from England) and by Rickes and others (from the United States). Its structure was discovered by Hodgkin; however, Minot and Murphy in 1926 showed that eating large amounts of liver could help correct pernicious anemia associated with deficiency of the vitamin. It took about two decades to identify the vitamin in liver.

SOURCES

The only dietary sources of vitamin B₁₂ for humans are animal products, which have derived their cobalamins from microorganisms. All naturally occurring vitamin B₁₂ is produced by microorganisms. Any vitamin B₁₂ found in plant foods probably could be traced either to contamination with microorganisms contained in manure or, in the case of legumes, to the presence of nitrogen-fixing bacteria in the plant root nodules [1]. Contaminated hands taking foods to the mouth may also provide vitamin B₁₂.

The best sources of the cobalamins are meat and meat products, poultry, fish, shellfish (especially clams and oysters), and eggs (especially the yolk); the cobalamins in these products are predominantly adenosyl- and hydroxocobalamin. Milk and milk products such as cheese, cottage cheese, and yogurt contain less of the vitamin, mainly as methyl- and hydroxocobalamins [2,3]. Cyanocobalamin may be

found in a few foods as well as in tobacco. Plant-derived foods are sometimes fortified with the vitamin. Cyanocobalamin and hydroxocobalamin are the forms commercially available in, for example, vitamin preparations. Within the body, cyanocobalamin is converted to aquo- or hydroxocobalamin, among other forms.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

The digestion and absorption of vitamin B₁₂ are believed to proceed according to the scheme depicted in Figure 9.33. Ingested cobalamins must be released from the proteins/polypeptides to which they are linked in foods. This release usually occurs through the actions of the gastric proteolytic enzyme pepsin and hydrochloric acid in the stomach.

Next, vitamin B₁₂ binds to an R protein. The cobalamin-binding R protein, found in saliva and gastric juice, binds to the vitamin prior to or after its release from food proteins. R proteins, known collectively as **cobalophilins** or **haptocorrins** (HCs), have a high affinity for cobalamins. The R protein typically binds to the vitamin as it is emptied from the stomach into the duodenum, the proximal or upper region of the small intestine. R proteins (or other factors secreted by pancreatic juice) are also thought to protect vitamin B₁₂ from bacterial use [4]. Within the duodenum, the R protein is hydrolyzed by pancreatic proteases, and free cobalamin is released. After release from the R protein, vitamin B₁₂ (all forms) binds to intrinsic factor (IF), a glycoprotein that is synthesized by gastric parietal cells but escapes the catabolic action of the proteases.

Several conditions can interfere with these just-described steps in the absorptive process. Pancreatic insufficiency is known to interfere with the release of cobalamin from the R protein and thus reduce vitamin absorption. Zollinger-Ellison syndrome results in increased gastric acid secretion, and with the increased acid, the pH of the digestive juices in the small intestine is lowered. This acidification can impair release of the vitamin from IF and thus inhibit absorption. These conditions among others increase a person's risk for deficiency and are discussed in more detail under the section on deficiency.

The cobalamin-IF complex travels from the duodenum to the ileum, where receptors (called cubilins) for vitamin B₁₂ are present and allow for absorption [5]. The vitamin is absorbed throughout the ileum, especially the distal third [1]. Although the cobalamin absorption process is not completely understood, absorption of the cubilin-IF-vitamin B₁₂ complex into the enterocyte is thought to occur by receptor-mediated endocytosis. A protein, megalin, is also thought to bind to the complex and to play a role in its transport into the cell. Within the enterocyte, B₁₂ is released from the IF

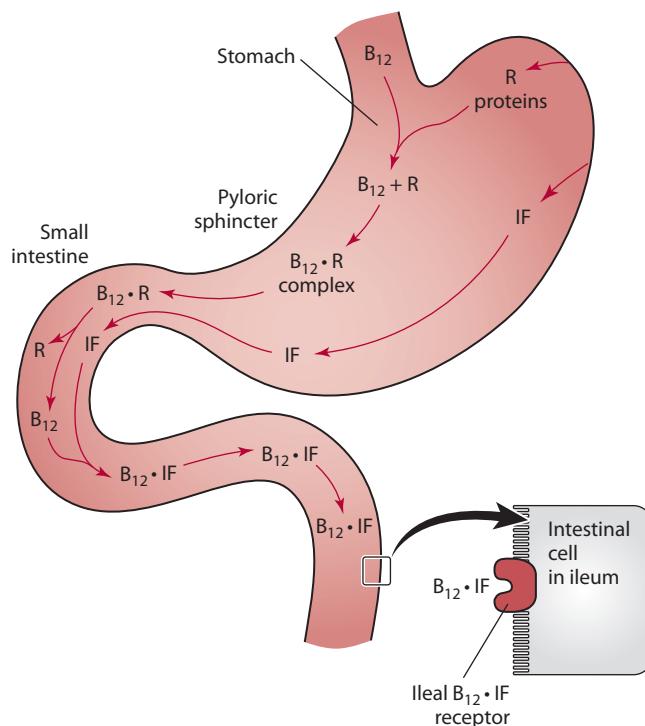


Figure 9.33 Vitamin B₁₂ absorption.

complex. In or before it is transported across the basolateral membrane of the ileal cells, vitamin B_{12} binds to transcobalamin II for transport in portal blood.

Although most vitamin B_{12} is absorbed by the process previously described, about 1% to 3% of intake is thought to be absorbed by passive diffusion, especially when pharmacological doses of vitamin B_{12} are ingested. Overall absorption of vitamin B_{12} from the diet ranges from about 11% to 65%, with decreased absorption efficacy as intake increases; in calculating recommendations for dietary B_{12} intakes, 50% absorption was assumed [6]. Absorption of free cyanocobalamin in people with healthy gastrointestinal tracts does not diminish with aging [7–10].

Enterohepatic circulation is important in vitamin B_{12} nutriture, accounting in part for the long biological half-life of cobalamin. About 1.4 μg (1.85 nmol) cobalamin per day is thought to be secreted into the bile [11]. With enterohepatic circulation, much of this cobalamin in the bile and in other intestinal secretions can bind to IF in the small intestine and be reabsorbed in the ileum. Thus, malabsorption syndromes not only decrease absorption of ingested cobalamin but also interfere with enterohepatic circulation, thereby increasing the amount of vitamin B_{12} required to meet body needs.

Following absorption of vitamin B_{12} , the vitamin appears in circulation about 3 to 4 hours later. Peak levels of the vitamin in the blood typically are not reached for another 4 to 8 hours. In the blood, methylcobalamin comprises about 60% to 80% and adenosylcobalamin perhaps up to 20% of total plasma cobalamin. Other forms of cobalamin in the blood include cyanocobalamin and hydroxocobalamin.

Vitamin B_{12} is found in the blood bound to one of three transcobalamins (TC), designated TCI, TCII, or TCIII. Whether attachment to TC occurs within the enterocyte or at the serosal surface is not known. The transcobalamins are considered R proteins. TCII is the main protein that carries newly absorbed cobalamin, in a one-to-one ratio, to the tissues. TCII is made in a variety of body cells. About 20% of cobalamin is transported on TCII, which as holo TCII has a half-life of under 2 hours. The exact functions of TCI and TCIII are unknown. TCIII may function in the delivery of cobalamin from peripheral tissues back to the liver. Up to about 80% of vitamin B_{12} is bound to TCI (also called haptocorrin), which may function as a circulating storage form of the vitamin and may prevent bacterial use of the vitamin. Cells are thought to take up the holoTCI B_{12} complex by nonspecific receptors after the protein has been desialylated.

Uptake of vitamin B_{12} into tissues is receptor dependent. All tissues appear to have receptors for TCII. The TCII-cobalamin complex is thought to bind to TCII receptors on cells. The complex is then taken into cells by endocytosis and fused to lysosomes that provide for the degradation of TCII and release of the vitamin within the cell cytosol. Genetic mutations in TCII have been documented. One fairly

common mutation is the substitution of cytosine (C) for guanine (G) at base pair 776 (written as TC 766C → G). This substitution results in the insertion of arginine instead of proline into TCII, and in turn diminishes the protein's (TCII) ability to bind and transport B_{12} to tissues. An estimated 20% of the population is homozygous for the GG variant. Moreover, studies have found that women with the GG genotype exhibit significantly lower serum vitamin B_{12} concentrations and higher serum homocysteine concentrations (a risk factor for heart disease) than those without the mutation [12].

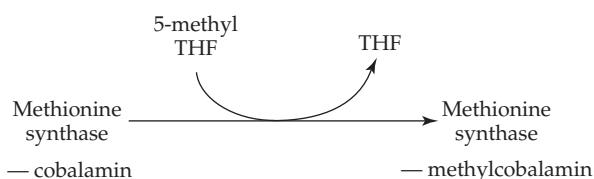
Metabolism of the various forms of the vitamin occurs within cells. Hydroxocobalamin, for example, may undergo cytosolic methylation to generate methylcobalamin or may undergo reduction and subsequent reaction with ATP in the mitochondria to yield adenosylcobalamin [1].

Vitamin B_{12} , unlike other water-soluble vitamins, can be stored and retained in the body for long periods of time, even years. About 2 to 4 mg of the vitamin is stored in the body, mainly (~50%) in the liver. Small amounts also are found in the muscle, bone, kidneys, heart, brain, and spleen and circulating in the blood as transcobalamins. Adenosylcobalamin represents about 70% of the body's vitamin B_{12} and is the primary storage form of the vitamin in the liver, red blood cell, kidney, and brain. Methylcobalamin is the main form of the vitamin in the blood. Hydroxocobalamin and methylcobalamin are also stored, but to a lesser extent.

FUNCTIONS AND MECHANISMS OF ACTION

Two enzymatic reactions requiring vitamin B_{12} have been recognized in humans. One of these reactions requires methylcobalamin, whereas the other must have adenosylcobalamin. Adenosyl- and methylcobalamin are formed by a complex reaction sequence (see [13]) resulting in the production of a carbon-cobalt bond between the cobalt nucleus of the vitamin and either the methyl or the 5'-deoxyadenosyl ligand.

The reaction requiring methylcobalamin as a coenzyme is the conversion of homocysteine into methionine (Figure 9.30). This reaction occurs in the cytoplasm of the cell and is shown below in a two-step process to facilitate understanding of the sequential nature of the reaction. First, to form the methylcobalamin needed in methionine synthesis, cobalamin bound to the methionine synthase (also called homocysteine methyltransferase) picks up the methyl group from 5-methyl tetrahydrofolate (THF), forming methylcobalamin bound to methionine synthase and THF.



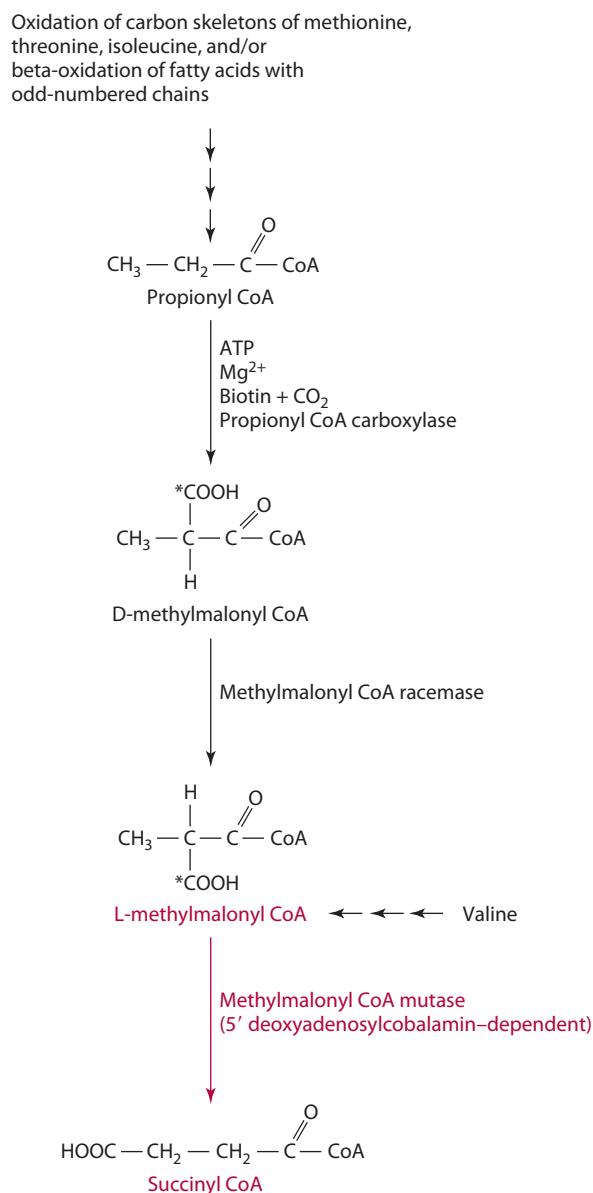
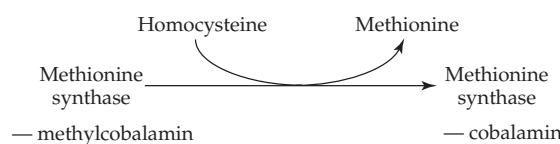


Figure 9.34 Role of vitamin B₁₂ in oxidation of L-methylmalonyl CoA.

Next, the methylcobalamin bound to methionine synthase releases the methyl group for transfer to homocysteine, producing methionine and cobalamin.



Because the formation of 5-methyl THF is irreversible, a vitamin B₁₂ deficiency traps body folate in the 5-methyl form, in what is known as the methyl-folate trap hypothesis. This hypothesis helps explain in part the synergism between folate and vitamin B₁₂ (see “Interactions with Other Nutrients” under “Folate”).

The second of the vitamin B₁₂-dependent reactions requires adenosylcobalamin. This reaction is catalyzed by a mutase and occurs in the mitochondria. Specifically, adenosylcobalamin is needed for methylmalonyl CoA mutase, which converts L-methylmalonyl CoA to succinyl CoA (Figure 9.34). L-methylmalonyl CoA is made from D-methylmalonyl CoA, which in turn is generated from propionyl CoA. Propionyl CoA arises from the oxidation of methionine, isoleucine, and threonine and of odd-chain fatty acids. The conversion of propionyl CoA to D-methylmalonyl CoA is an ATP-, Mg²⁺-, and biotin-dependent reaction (previously discussed in the section on biotin) (Figure 9.24). Methylmalonyl CoA mutase (a dimer) requires two adenosylcobalamin molecules (one per subunit) to convert L-methylmalonyl CoA to succinyl CoA, the TCA cycle intermediate (Figure 9.34). With a deficiency of vitamin B₁₂, mutase activity is impaired and methylmalonyl CoA and methylmalonic acid, formed from hydrolysis of methylmalonyl CoA, accumulate in body fluids. Genetic defects in methylmalonyl CoA mutase and adenosylcobalamin synthesis have also been demonstrated and result in the accumulation of methylmalonyl CoA and methylmalonic acid. The response of serum methylmalonic acid to vitamin B₁₂ depletion and repletion is useful in the diagnosis of vitamin B₁₂ deficiency and response to treatment.

METABOLISM AND EXCRETION

Vitamin B₁₂ is not extensively (if at all) degraded prior to excretion. Turnover of the vitamin is approximately 0.1% per day, with most of the vitamin excreted, bound to R protein, in the bile. Little urinary excretion occurs, but trace dermal losses of vitamin B₁₂ occur.

RECOMMENDED DIETARY ALLOWANCE

Recommendations for vitamin B₁₂ intake are based on estimates of vitamin intake and turnover and on amounts of the vitamin needed for the maintenance of normal serum indices of the vitamin and hematological status. The RDA for adults for vitamin B₁₂ is 2.4 µg per day, with increases of 0.2 µg and 0.4 µg per day suggested for women during pregnancy and lactation, respectively [6]. The requirement for the vitamin for adults is 2.0 µg /day [6]. People age 51 years and older are counseled to consume foods fortified with the vitamin or consume vitamin B₁₂ supplements, because 10% to 30% of older people have changes to the gastrointestinal tract that limit their ability to absorb food-bound forms of the vitamin [6]. The inside cover of the book provides additional recommendations for vitamin B₁₂ intake for other age groups.

DEFICIENCY: MEGALOBLASTIC MACROCYTIC ANEMIA

Deficiency of vitamin B₁₂, like that of folate, results in megaloblastic macrocytic anemia. Manifestations of vitamin B₁₂ deficiency occur in stages. Initially, serum vitamin B₁₂ concentrations diminish; serum B₁₂ concentrations, however, may remain normal until stores of the vitamin become depleted. Second, cell concentrations of the vitamin diminish. Third, DNA synthesis decreases and serum homocysteine and methylmalonic acid concentrations increase. Finally, morphological and functional changes occur in blood cells and in precursor blood cells in bone marrow resulting in a macrocytic megaloblastic (large immature cell) anemia [14]. Most deficiency signs and symptoms are of neurologic and hematologic origin; some signs and symptoms include skin pallor, fatigue, shortness of breath, palpitations, insomnia, tingling and numbness (paresthesia) in extremities, abnormal gait, loss of concentration, memory loss, disorientation, swelling of myelinated fibers, and possibly dementia. Neurological problems occur in about 75% to 90% of deficient people [6,15].

The megaloblastic macrocytic anemia associated with a vitamin B₁₂ deficiency is detailed under both “Folate” and “Vitamin B₁₂ (Cobalamin),” because deficiencies of both result in megaloblastic macrocytic anemia. In fact, megaloblastic anemia related to vitamin deficiency can be corrected with large doses of folate. However, the neuropathy, characterized by demyelination of nerves, caused by a lack of vitamin B₁₂ is not responsive to folate therapy. The neuropathy is usually found in vitamin B₁₂-deficient people with decreased activity of both vitamin B₁₂-dependent enzymes. Its cause may be related to the availability of methionine [16,17]. The neuropathy can be ameliorated through increased exogenous methionine or an accelerated production of methionine from homocysteine, a reaction that requires vitamin B₁₂. An inadequate amount of methionine caused by a deficiency of vitamin B₁₂ decreases the availability of S-adenosyl methionine (SAM). Remember that SAM is required for methylation reactions, which are essential to myelin maintenance and thus neural function. SAM deficiency in the nervous system (i.e., cerebrospinal fluid) has been implicated in the pathogenesis of cobalamin neuropathy [18].

In addition to anemia, plasma vitamin B₁₂ concentrations are inversely associated with plasma homocysteine concentrations [19–24]. Elevated plasma homocysteine concentrations are considered a risk factor for coronary heart disease, and concentrations may be lowered with vitamin B₁₂, folate, and vitamin B₆ supplements [19,23–25]. This relationship among vitamin B₁₂, folate, vitamin B₆, plasma homocysteine concentrations, and heart disease is discussed in further detail in the “Folate” section of this chapter.

Inadequate absorption of the vitamin, rather than inadequate dietary intake, causes most of the vitamin B₁₂ deficiency seen in the United States. However, a strict vegetarian diet can produce a deficiency of the vitamin fairly quickly in an infant or a young child with minimal stores of the vitamin. In contrast to children, adults consuming animal-based diets who switch to strict vegetarian diets without consuming vitamin-fortified foods may not develop clinical symptoms of deficiency for decades, because they have accumulated fairly large stores of the vitamin. Inadequate vitamin B₁₂ absorption can result from any of several problems and is especially prevalent in older people. Malabsorption can result from pernicious anemia, an autoimmune condition in which the body produces antibodies that attack gastric parietal cells and thereby diminish IF production. Remember, IF is required to absorb the vitamin. Other conditions that can impair absorption of the vitamin include those causing a lack of IF, such as atrophic gastritis (loss and inflammation of gastric cells) or gastrectomy (removal of all or a portion of the stomach). In some forms of atrophic gastritis, antibodies are made against the proton pump in gastric parietal cells [26]. Diminished hydrochloric acid release (**achlorhydria**) also decreases release of the vitamin bound to food, causing malabsorption of the vitamin. People with a decreased absorptive surface in the ileum, such as occurs with ileal resection, celiac and tropical sprue, ileitis (inflammation of the ileum) also malabsorb the vitamin and are at risk for deficiency. People with Zollinger-Ellison syndrome produce excessive quantities of gastric acid, which results in increased acid release both into the stomach and passed into the small intestine with the chyme. The increased acid in the small intestine lowers the intestinal pH and is thought to impair the release of the B₁₂ from the R-protein and the binding of the vitamin to IF. In addition, people with parasitic infections such as tapeworms may develop a vitamin B₁₂ deficiency because the parasite uses the vitamin, and consequently its availability to the infected person is limited. Prolonged use of some medications, such as H₂ blockers and proton pump inhibitors used to treat people with ulcers or gastroesophageal reflux disease (GERD), also is associated with diminished absorption of vitamin B₁₂. Bacterial overgrowth occurs because of the higher intestinal pH (less acid is produced, because of the medications), and the bacteria use the vitamin B₁₂ and thus limit its availability. Finally, people who have poor vitamin B₁₂ status may exhibit deterioration of nervous system function, especially demyelinating problems, following nitrous oxide anesthesia [27–29]. Nitrous oxide (an anesthetic agent) has been shown to inhibit the activity of methionine synthase by reacting with methylcobalamin and possibly altering the oxidation state of the cobalt (from 1+ to 3+) [1].

The incidence of vitamin B₁₂ deficiency in the elderly may be as high as 15%, and the vitamin B₁₂ content of multivitamin preparations is usually not sufficient for

treatment [9,30]. Oral vitamin B₁₂ in amounts of at least 6 to 9 µg and possibly up to 300 µg appears to be necessary to correct deficiency in the elderly [30]. Treating pernicious anemia or deficiency secondary to malabsorption often requires monthly intramuscular injection of the vitamin in amounts of 500 to 1,000 µg [6] or oral ingestion of pharmacologic amounts (2 mg) of the vitamin [31]. Vitamin B₁₂ nasal sprays also are available. NascoB[®], for example, provides the vitamin as cyanocobalamin (500 µg/spray) in a nasal spray that is beneficial to people with malabsorptive disorders such as inflammatory bowel disease or those with pernicious anemia.

TOXICITY

Although no clear toxicity from massive doses of vitamin B₁₂ has ever been recorded, neither has any benefit been noted from an excessive intake of the vitamin by people with adequate vitamin status [6]. No tolerable upper intake level for vitamin B₁₂ has been established [6].

ASSESSMENT OF NUTRITURE

Vitamin B₁₂ status may be assessed using several indices. Serum vitamin B₁₂ concentrations, which include cobalamin bound to TCI, TCII, and TCIII, are commonly measured and reflect both intake and status. Concentrations in the serum of <200 pg/mL (based on a radioassay method) are considered deficient [6]. Although, because serum vitamin B₁₂ concentrations can be maintained at the expense of tissues, a person may exhibit normal serum concentrations but have low tissue concentrations [15]. Thus, assessment that includes measuring indices, in addition to serum concentrations, is beneficial.

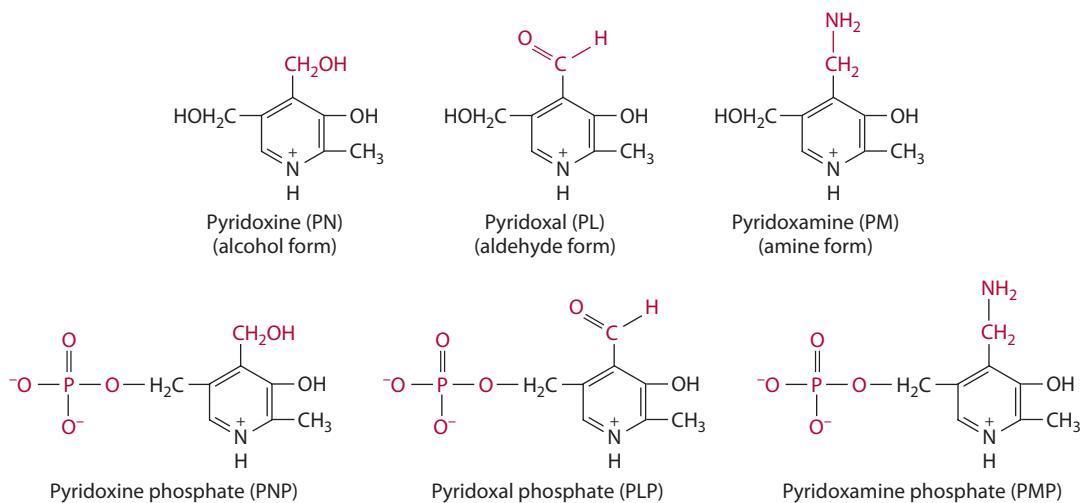
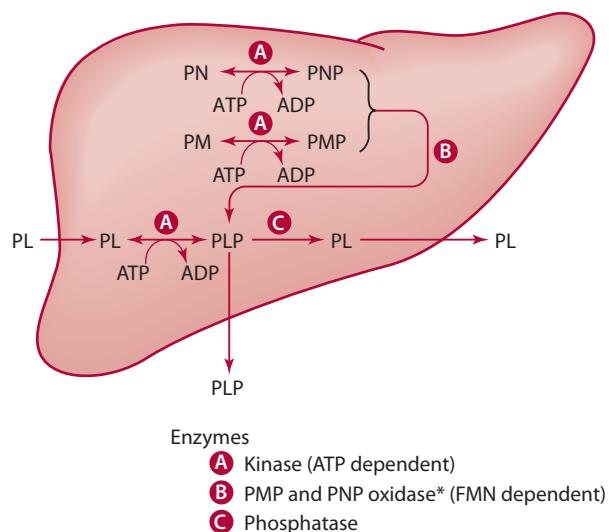
Measurements of serum methylmalonyl CoA or methylmalonic acid and of homocysteine also are used to assess vitamin B₁₂ status. Increased concentrations of methylmalonyl CoA (or methylmalonic acid) and of homocysteine, substrates normally metabolized by vitamin B₁₂-dependent enzymes, occur with diminished enzyme activity resulting from insufficient vitamin B₁₂. Increased blood or urinary excretion of methylmalonic acid with deficient vitamin B₁₂ status and response (decreases) of blood methylmalonic acid concentrations to vitamin B₁₂ supplementation are helpful indicators of vitamin status [31]. Normally, no or only trace amounts of methylmalonic acid are excreted in the urine; however, with vitamin B₁₂ deficiency, methylmalonic acid excretion may exceed 300 mg per day [15]. Reticulocyte counts also increase within 48 hours following vitamin B₁₂ supplementation of deficient people.

Other tests used to assess vitamin B₁₂ nutriture include the deoxyuridine suppression test, discussed previously under “Assessment of Nutriture” in the folate section, and

the Schilling test, which is used to determine problems of vitamin B₁₂ absorption related to IF insufficiency. The Schilling test involves orally administering radioactive vitamin B₁₂ and measuring urinary excretion of the vitamin over various times. Below-normal urinary excretion of the vitamin suggests impaired absorption.

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**Figure 9.35** Vitamin B₆ structures.

*Oxidase is found mainly in the liver and enterocytes.

Figure 9.36 Vitamin B₆ metabolism in the liver.

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Vitamin B₆

Vitamin B₆ exists as several vitamers, the structural formulas of which are given in Figure 9.35. These vitamers are interchangeable and comparably active (Figure 9.36). Pyridoxine represents the alcohol form, pyridoxal the aldehyde form, and pyridoxamine the amine form. Each has a 5'-phosphate derivative.

The vitamin was identified in 1934 and its structure confirmed in 1938. Some of the initial research was aimed at correcting dermatitis in rats. Kuhn and Szent-Györgyi are credited with isolating the vitamin (which was called vitamin B₆) to correct the dermatitis in 1938. The pyridoxal and pyridoxamine forms of the vitamin were identified in the mid-1940s.

SOURCES

All B₆ vitamers are found in food. Pyridoxine, the stablest of the compounds, and its phosphorylated form are found almost exclusively in plant foods. In some plants, vitamin B₆ is found in a conjugated form, pyridoxine-glucoside. Pyridoxal phosphate and pyridoxamine phosphate are found primarily in animal products, with sirloin steak, salmon, and the light meat of chicken being rich sources [1,2]. Excellent sources of vitamin B₆ in commonly

consumed foods include meats, whole-grain products, vegetables, some fruits (e.g., bananas), and nuts. Fortified cereals also represent a major contributor of vitamin B₆ in the diet. Vitamin B₆ in supplements is generally found as pyridoxine hydrochloride.

The bioavailability of vitamin B₆ from different food sources is influenced by the food matrix and by the extent and type of processing to which the foods are subjected. Much of the vitamin originally present in foods can be lost through processing, including, for example, heating, sterilizing, canning, and milling, with fewer losses occurring during storage and handling [1–3].

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

For vitamin B₆ to be absorbed, the phosphorylated vitamers must be dephosphorylated. Alkaline phosphatase, a zinc-dependent enzyme found at the intestinal brush border, or other intestinal phosphatases hydrolyze the phosphate to yield either pyridoxine (PN), pyridoxal (PL), or pyridoxamine (PM).

PL, PN, and PM are absorbed primarily in the jejunum by passive diffusion. At physiological intakes, the vitamin is absorbed rapidly in its free form; however, when the phosphorylated vitamers are ingested in high concentrations, some of these compounds may be absorbed per se [3]. Absorption of some pyridoxine glucosides may also occur by passive diffusion; mucosal glucosidase can hydrolyze the glucosides to varying degrees [4]. Overall absorption of vitamin B₆ furnished by the average U.S. diet is about 75%, with a range of about 61% to 92% [5,6].

Little metabolism of the vitamin occurs within the intestinal cell. Most PN, PL, and PM is released unchanged into portal blood and taken up by the liver, where it is converted primarily to PLP. PLP is the main (60% to 90% of the total) form of the vitamin found in systemic blood. Most PLP is bound to albumin. Other forms of the vitamin present in blood include PL, PN, PM, and PMP. From the plasma, the unphosphorylated vitamers may be taken up by red blood cells, converted to PLP, and bound to hemoglobin.

The liver is the main organ that takes up (by passive diffusion) and metabolizes newly absorbed vitamin B₆. The liver stores about 5% to 10% of the vitamin [7]. Muscles represent the major (75%–80%) storage site for the vitamin, which is found in the body in amounts totaling about 165 mg [8]. Most vitamin B₆ occurs in muscle as PLP bound to glycogen phosphorylase [5,8,9]. Phosphorylation of the vitamin prevents its diffusion out of the cell, and the binding of the vitamin to protein prevents hydrolysis by phosphatases.

Most vitamin B₆ metabolism occurs in the liver. Unphosphorylated forms of the vitamin typically are phosphorylated by a kinase using ATP within the cytoplasm of

the hepatocyte (liver cell), as shown in Figure 9.36. PNP and PMP are then generally converted by the action of an FMN-dependent oxidase to PLP. Thus, vitamin B₆ metabolism is dependent on adequate riboflavin status.

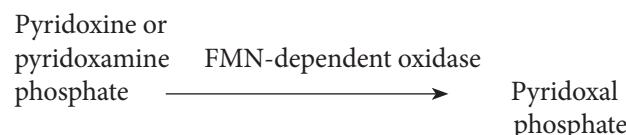


Figure 9.36 depicts the interconversion of the B₆ vitamers. Intracellular PLP concentrations are dependent, in part, on the availability of binding proteins. With saturation of binding proteins, unbound PLP is hydrolyzed to PL, which is released into the blood for use by other tissues. From the liver, mostly PLP and PL, with smaller amounts of the other vitamers, are released for transport to extrahepatic tissues.

Only unphosphorylated forms of the vitamin are taken up by tissues, thus, PLP in the blood is hydrolyzed by alkaline phosphatase prior to cellular uptake. Within the cell, PL is phosphorylated by pyridoxine kinase in an ATP-dependent reaction. Pyridoxine kinase is found in almost all tissues, and phosphorylation traps the vitamin in the cells. Most tissues, however, lack sufficient oxidase to convert PNP and PMP into the coenzyme form PLP. PNP/PMP oxidase is found mainly in the liver and intestine and to lesser extents in the muscle, kidney, brain, and red blood cells.

FUNCTIONS AND MECHANISMS OF ACTION

The coenzyme form of vitamin B₆, PLP, is associated with a vast number (>100) of enzymes, the majority of which are involved in amino acid metabolism. Some noncoenzyme roles of the vitamin affect the action of steroid hormones and gene expression.

Coenzymes

As a coenzyme in reactions involving amino acids, PLP, through the formation of a Schiff base (the product formed by an amino group and an aldehyde), labilizes all the bonds around the α-carbon of the amino acid. The specific bond that is broken is determined by the catalytic groups of the particular enzyme to which PLP is attached. The covalent bonds of an α-amino acid that can be made labile by its binding to specific PLP containing enzymes are shown in Figure 9.37.

Some of the reactions involving amino acids that are catalyzed by PLP include transamination (which can also be catalyzed by PMP), decarboxylation, transulfhydration and desulfhydration, dehydration (elimination)/deamination, cleavage, racemization, and synthesis. In addition to its participation in reactions involving amino acids, vitamin B₆ functions by a different mechanism in the initial

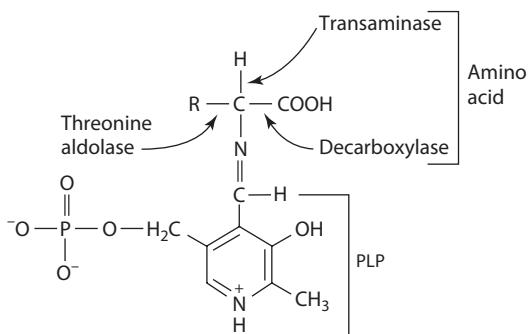


Figure 9.37 The covalent bonds of an α acid that can be made labile by its binding to PLP-containing enzymes.

step of glycogen metabolism. Each of these reactions is discussed briefly.

Transamination Of particular importance are the transamination reactions in which PMP as well as PLP can serve as a coenzyme. The most common aminotransferases for which PLP (or PMP) is a coenzyme are glutamate oxaloacetate transaminase (GOT, also called aspartic amino transferase or AST or AsAT) and glutamate pyruvate transaminase (GPT, also called alanine aminotransferase or ALT or AlAT) (Figure 6.25). Figures 9.38a and b show the two phases of transamination and demonstrate how PLP forms a Schiff base. In the first phase, the corresponding α -keto acid of the amino acid is produced along with PMP. In the second phase, the transamination cycle is completed as a new α -keto acid substrate receives the amino group from the PMP. The corresponding amino acid is generated, along with regeneration of PLP.

Decarboxylation Decarboxylation reactions involve the removal of the carboxy (COO^-) group from an amino acid or other compound. Some examples of some common decarboxylation reactions include the formation of γ -aminobutyric acid (GABA) from glutamate (Figure 6.43), the production of serotonin from 5-hydroxytryptophan (Figure 6.41), and the synthesis of histamine from the amino acid histidine (Figure 6.33). Dopamine is formed following decarboxylation of dihydroxyphenylalanine (also known as L-dopa), which is generated from the amino acid tyrosine (Figure 6.28).

Transulfhydration and Desulfhydration PLP is required for transulfhydration reactions in which cysteine is synthesized from methionine (Figure 6.30). Both cystathione synthase and cystathione lyase require PLP. Cysteine undergoes desulfhydration followed by transamination to generate pyruvate.

Dehydration (also called elimination) or Deamination PLP also is sometimes involved in dehydration or elimination reactions when an amino group (NH_2) is removed from a

compound such as an amino acid and released as ammonia or ammonium. Threonine dehydratase, for example, is a PLP-dependent enzyme that removes water and the amino group from the amino acid threonine (Figure 6.24).

Cleavage An example of a cleavage reaction requiring PLP is the removal of the hydroxymethyl group from serine. In this reaction, PLP is the coenzyme for a transferase that transfers the hydroxymethyl group of serine to tetrahydrofolate (THF) so that glycine is formed (Figure 9.28).

Racemization PLP is required by racemases that catalyze the interconversion of D- and L-amino acids. Although such reactions are more prevalent in bacterial metabolism, some occur in humans.

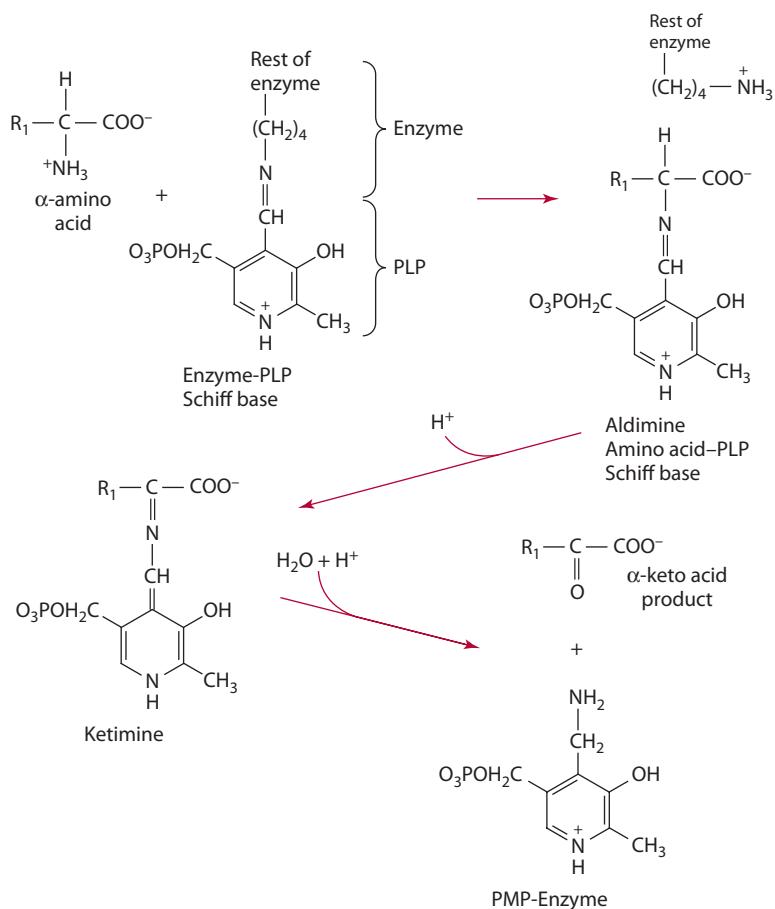
Other Synthetic Reactions Vitamin B₆ is also necessary as a coenzyme in the first step in the synthesis of heme (Figure 12.5). PLP is required for δ -aminolevulinic acid synthetase, which catalyzes the condensation, followed by decarboxylation, of glycine with succinyl CoA to form δ -aminolevulinic acid (ALA) in the mitochondria of the cell. ALA moves into the cytosol of the cell, where it is used to synthesize porphobilinogen (PBG), the parent pyrrole compound in porphyrin synthesis. Through a series of reactions, PBG is converted into protoporphyrin IX, which with the addition of Fe²⁺ by ferrochelatase forms heme.

PLP functions as a cofactor for another condensation reaction necessary for sphingolipid synthesis. Specifically, the amino acid serine condenses with palmitoyl CoA in a reaction catalyzed by a PLP-dependent transferase to form 3-dehydrosphinganine. This latter compound serves as a precursor for sphingolipids.

Niacin (NAD) synthesis from tryptophan also requires an important PLP-dependent reaction. Specifically, kynureninase required for the conversion of 3-hydroxykynurene to 3-hydroxyanthranilate requires vitamin B₆ (PLP) as a coenzyme (Figure 9.15).

Other compounds synthesized in the body in vitamin B₆-dependent reactions include carnitine, a nonprotein nitrogen-containing compound required for fatty acid oxidation (Figure 6.12), and taurine, a neuromodulatory compound generated from cysteine metabolism (Figure 6.30).

Glycogen Degradation The function of PLP in glycogen degradation is poorly understood. Glycogen is catabolized by glycogen phosphorylase to form glucose 1-PO₄ (Figure 3.15); vitamin B₆ is required for glycogen phosphorylase activity. The mechanism of action of the coenzyme appears to be different from that exerted with other enzymes. The phosphate of the coenzyme is believed to be involved as a proton buffer to stabilize the compound and permit covalent bonding of the phosphate to form glucose 1-PO₄ [10]. Most vitamin B₆ found



(a)

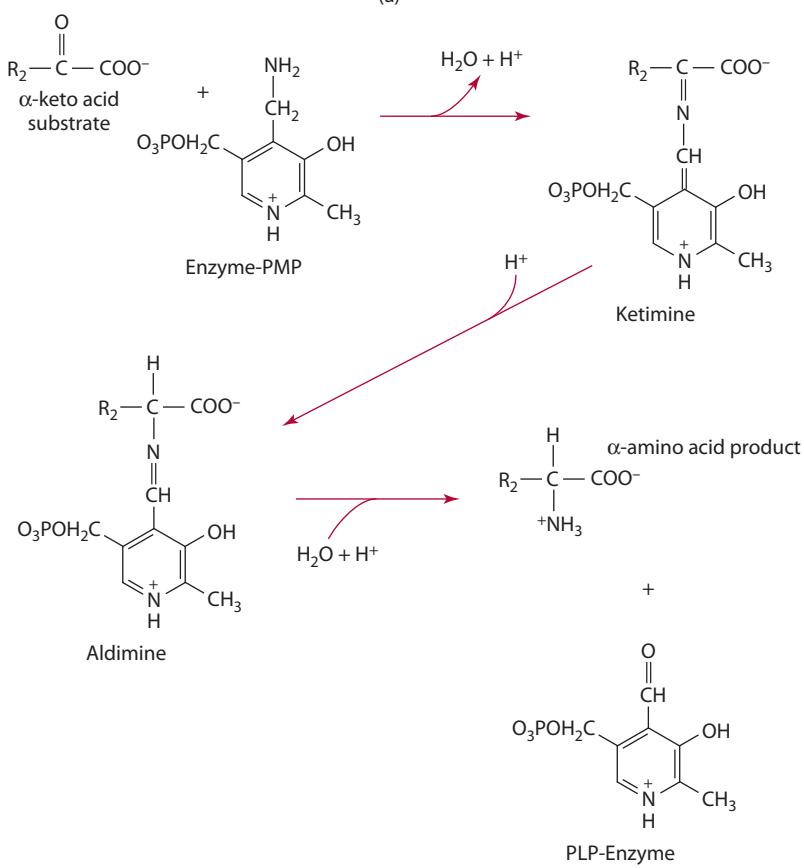


Figure 9.38 (a) The role of vitamin B_6 in transamination, phase 1. (b) The role of vitamin B_6 in transamination phase 2.

in muscle is present as PLP, which in turn is bound to glycogen phosphorylase.

Noncoenzyme Role: Steroid Hormone Action

Although the coenzyme roles of vitamin B₆ have been more thoroughly investigated, the vitamin also appears to moderate the effects of some steroid hormones. Vitamin B₆ as PLP has been shown to react with lysine residues in steroid hormone receptor proteins to prevent or interfere with hormone binding. These receptor proteins mediate nuclear uptake of steroid hormones and the interaction of the nucleoproteins with the DNA [7]. Thus, vitamin B₆ appears to be able to diminish the actions of steroids. Diminishing the action of, for example, glucocorticoid hormones can in turn influence metabolism of protein, carbohydrate, and lipid.

METABOLISM AND EXCRETION

Little vitamin B₆ is excreted in the feces [5,11]. 4-pyridoxic acid (PIC) is the major metabolite of the vitamin and results from the oxidation of PL by either NAD-dependent aldehyde dehydrogenase, found in all tissues, or FAD-dependent aldehyde oxidases, found in the liver and kidneys. 4-pyridoxic acid is excreted in the urine and indicates recent vitamin intake, not vitamin stores [5]. Ingesting large doses (100 mg) of the vitamin as PN results in urinary excretion of PN intact and 5-pyridoxic acid and lower urinary 4-pyridoxic acid excretion. It appears that when PN is administered at high levels, the kidney tubules reduce plasma content of the vitamer by excreting some of it into the urine [5,11].

RECOMMENDED DIETARY ALLOWANCE

The 1998 RDA for vitamin B₆ for adult men age 19 to 50 years is 1.3 mg per day (requirement 1.1 mg) and for men age 51 years and older, 1.7 mg per day (requirement 1.4 mg) [5]. For adult women age 19 to 50 years, the RDA for vitamin B₆ is also 1.3 mg per day (requirement 1.1 mg), and for women age 51 years and older it is 1.5 mg daily (requirement 1.3 mg) [5]. With pregnancy and lactation, recommendations for vitamin intake increase to 1.9 mg and 2.0 mg, respectively [5]. Recommendations are based largely on maintenance of adequate plasma concentrations (at least 20 nmol/L) of the vitamin [5]. Some have suggested the recommendations need to be raised [12,13]. The inside covers of the book provide additional recommendations for vitamin B₆ for other age groups.

DEFICIENCY

Vitamin B₆ deficiency is relatively rare in the United States. In the 1950s, deficiency occurred in infants

because of severe heat treatment of infant milk. The heat processing resulted in a reaction between the PLP and the ε amino group of lysine in the milk proteins to form pyridoxyl-lysine, which possesses little vitamin activity. Signs of vitamin B₆ deficiency include sleepiness, fatigue, cheilosis, glossitis, and stomatitis in adults, and neurological problems such as abnormal EEGs, seizures, and convulsions in infants. A hypochromic, microcytic anemia may also result from a vitamin B₆ deficiency due to impaired heme synthesis. Deficiency also alters calcium and magnesium metabolism, impairs niacin synthesis from tryptophan, and inhibits metabolism of homocysteine. The last results in hyperhomocysteinemia, a risk factor for heart disease [14].

Groups particularly at risk for vitamin B₆ deficiency are the elderly, who have a poor intake of the vitamin and may also have accelerated hydrolysis of PLP and oxidation of PL to PIC; people who consume excessive amounts of alcohol (alcohol can impair conversion of PN and PM to PLP, and the presence of acetaldehyde formed from ethanol metabolism may enhance hydrolysis of PLP to PL with subsequent formation of the excretory product pyridoxic acid); and people on a variety of drug therapies, including isoniazid, penicillamine, corticosteroids, and anticonvulsants [1,2].

TOXICITY

Pharmacological doses of vitamin B₆ have been advocated to prevent or treat a variety of disease states, including hyperhomocysteinemia, carpal tunnel syndrome, premenstrual syndrome, depression, muscular fatigue, and paresthesia (tingling or numbness of the feet and hands) [15]. Although some beneficial results from megadoses of the vitamin have been noted in selected people, indiscriminate use of the vitamin is not without risk. Excessive pyridoxine use causes sensory and peripheral neuropathy [16]. Some symptoms include unsteady gait, paresthesia, and impaired tendon reflexes [17]. High intakes of pyridoxine also appear to cause degeneration of dorsal root ganglia in the spinal cord, loss of myelination, and degeneration of sensory fibers in peripheral nerves [13,15]. The tolerable upper intake level for vitamin B₆ is 100 mg per day for adults to minimize the development of neuropathy [5].

ASSESSMENT OF NUTRITION

Plasma PLP concentrations are thought to be the best indicator of vitamin B₆ tissue stores, with plasma PLP <20 nmol/L suggestive of vitamin deficiency, concentrations of 20–30 nmol/L suggestive of marginal status, and adequacy indicated by plasma concentrations >30 nmol/L [5,11]. Several other indices may be used in combination with plasma PLP concentration to assess

vitamin B₆ nutriture. A commonly used functional test measures xanthurenic acid excretion following tryptophan loading (2 g or 100 mg of tryptophan/kg body weight). Abnormally high xanthurenic acid excretion (>25 mg in 6 hours) is found in vitamin B₆ deficiency because 3-hydroxykynurenine, an intermediate in tryptophan metabolism, cannot lose its alanine moiety and be converted to 3-hydroxyanthranilate, as should occur in the liver (Figure 9.15). Instead, 3-hydroxykynurenine is converted to xanthurenic acid, which is excreted in the urine. Interpreting this test is sometimes difficult, owing to factors other than vitamin B₆ in tryptophan metabolism. Acceptable xanthurenic acid excretion following the tryptophan load is <25 mg/6 hours.

Urinary vitamin B₆ and 4-pyridoxic acid also have been used to assess the status of vitamin B₆. Urinary vitamin B₆ excretion measured over several 24-hour urine collections for a period of 1 to 3 weeks is recommended to more accurately assess vitamin B₆. Urinary 4-pyridoxic acid concentrations of $\leq 3.0 \mu\text{M}/\text{day}$ are thought to indicate deficiency [18]. Urinary 4-pyridoxic acid excretion is considered to be a short-term indicator of vitamin B₆ status, however, and cutoff values are controversial [5,11,18]. Urinary vitamin B₆ excretion alone or in comparison to creatinine excretion is also used. Urinary B₆ excretion of $<0.5 \mu\text{M}/\text{day}$ or $<20 \mu\text{g/g}$ creatinine suggest B₆ deficiency, whereas B₆ excretion $\geq 0.5 \mu\text{M}/\text{day}$ or $\geq 20 \mu\text{g/g}$ creatinine suggests acceptable vitamin B₆ status.

Measuring erythrocyte transaminase activity before and after adding vitamin B₆ is also useful in determining vitamin B₆ nutriture. However, because of a variety of limitations with the assays, these tests are better used as an adjunct to other tests. Erythrocyte transaminase index examines the activity of erythrocyte glutamic oxaloacetic transaminase (EGOT, also called aspartic amino transferase or EAST) after the addition of vitamin B₆. This assay and the assay discussed next are thought to represent long-term vitamin status. Deficient vitamin B₆ status is suggested by activity of >1.85 following the addition of the vitamin [18,19]. Similarly, activity of erythrocyte glutamic pyruvic transaminase (EGPT, also called alanine aminotransferase or EALT) of >1.25 suggests B₆ deficiency, whereas activity of <1.25 indicates adequate status [18,19].

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Genetics and Nutrition: The Possible Effect on Folate Needs and Risk of Chronic Disease

"Genomic medicine holds the ultimate promise of revolutionizing the diagnosis and treatment of many illnesses." [1]

Introduction

The results of the Human Genome Project have spawned a huge number of articles—both peer-reviewed and in the mass media—about the implications for individuals, families, and society [2, 3]. While the current results are most applicable to medicine, it is likely that *nutrigenomics* will grow and provide insight into individual nutrient needs [2, 4, 5]. Nutrigenomics studies the interaction and influence of nutrients on gene expression and resulting health outcomes. DeBusk summarizes that there are three effects of nutrients on the expression of our genes: (1) nutrients can complete gaps in our DNA, (2) nutrients can interact with DNA causing the synthesis of necessary proteins for the body, and (3) nutrients can interfere with the expression of genes that could produce harmful effects [2].

Nutrition professionals in the near future may need to both understand and practice nutrigenomics to provide individualized nutrition advice designed for their clients' and patients' unique genetic traits [5, 6]. Recently, the Commission on the Accreditation of Dietetic Education added genetics and nutrition to its list of required knowledge for the education of dietetic professionals [7]. By increasing our study and understanding of nutrigenomics, nutrition professionals will continue to be able to provide high-quality, science-based care.

Discoveries about folate metabolism provide an often-used example of nutrigenomics [8]. The identification of genetic variations in folate metabolism have been linked to neural tube defects, fetal malformations, coronary heart disease, colorectal cancer, dementia, and other health problems. The purpose of this Perspective is to (1) describe the most common types of genetic variants in 5,10-methylenetetrahydrofolate reductase, (2) review the prevalence of the genetic variation in different ethnic groups, and (3) review the research that links these variants to alterations in disease risk.

N⁵, N¹⁰ methylenetetrahydrofolate reductase and Its Genetic Variants

As shown in Figures 9.28 and 9.30, N⁵, N¹⁰ (also referred to as 5,10- without showing the N) methylene tetrahydrofolate reductase (MTHFR) catalyzes the unidirectional conversion of N⁵ N¹⁰ methylene THF

to N⁵ methyl THF. The activity of MTHFR, along with adequate amounts of NADPH and FADH₂, is essential to maintain appropriate levels of N⁵ methyl THF levels in the cell.

If MTHFR activity is low, intracellular N⁵ methyl THF will decrease and an impaired conversion of homocysteine to methionine would result. A lack of methionine results in a lack of methyl groups necessary for methylation reactions that include the synthesis of DNA, carnitine, creatine, epinephrine, purines, and nicotinamide. At the same time, an accumulation of homocysteine is widely believed to increase risk to cardiovascular disease and dementias. Clearly, impairing the formation of N⁵ methyl THF has an effect on the body's ability to synthesize methylated products and remove homocysteine.

Several genetic variations of MTHFR have been reported. These variants are caused by substitutions in the DNA sequence that codes for the enzyme. Since these variations are shared by more than 1% of the population, they are given the name *genetic polymorphisms* [8]. The genetic polymorphisms of MTHFR cause a decrease in its activity and the subsequent formation of N⁵ methyl THF. Two MTHFR polymorphisms have been reported [9]. These include a substitution of a thymine (abnormal) for a cytosine (normal) base at position 677 (called C677T) and a cytosine base (abnormal) for an adenine (normal) at position 1298 (called A1298C). These substitutions cause a molecule of valine to be inserted into MTHFR instead of alanine (C677T) or alanine instead of glutamate (A1298C) [9,10]. The polymorphism that is the most studied is the C677T, but characterizations of the A1298C have also occurred.

The C677T variant can be heterozygous or homozygous. An individual with the heterozygous genotype, abbreviated CT, received one normal and one abnormal allele, while the homozygous genotype is abbreviated TT or CC [2, 9]. The homozygous individual, who received two abnormal alleles, is abbreviated TT and has a lower MTHFR activity. The homozygous CC individual does not have an altered genotype and is considered to have a normal MTHFR activity. The individual with the TT genotype may have an increased plasma homocysteine level and a decrease in the methylation reactions described above. Research has identified that these individuals have increased hypomethylation of DNA, lower serum and RBC folate levels, and an increased risk to neural tube defects [11]. This increased risk to disease is less associated with the CT heterozygote, and most research occurs in the TT homozygote.

Like the C677T polymorphism, the A1298C variant exists in the homozygote normal-activity (AA), heterozygote (AC), and homozygote low-activity (CC).

Since these polymorphisms in MTHFR have been identified, many researchers have questioned the folate needs of individuals who do not have normal MTHFR activity and the effect on their individualized risk of chronic diseases. The identification of MTHFR polymorphisms has implications related to the individualization of nutrition care.

Ethnic Differences in Genetic Variations in MTHFR

Research reports indicate that these polymorphisms are distributed differently in ethnic groups. Esfahani, Cogger, and Caudill [10] reported that among a convenience sample of 433 women living in California, 18.1% of Mexican women had the C677T TT genotype, while this was true in only 7.2% of Caucasian, 3.8% of Asian, and 0% of African American women. The A1298C genotype was found in 7.9% of white women for the CC genotype, but was present in much lower percentages for all other ethnic groups. Further characterization found that 17.6% of Mexican women and 15.1% of white women had the homozygous low-activity genotype for both C677T and A1298C MTHFR. These findings differ from those reported by Botto and Yang [12] who stated that less than 10% of African Americans, over 40% of Italians and California Hispanics, and 22% of Norwegians had the C677T MTHFR genotype. Therefore, it is not clear exactly how these polymorphisms are distributed, but some ethnic groups are at greater risk than others.

While the literature often reports a differing risk to disease in those with the C677T homozygote genotype, it is not clear that these individuals have greater folate needs. Using a depletion/repletion design with Mexican women, Guinotte et al. [13] measured serum folate, RBC folate, plasma homocysteine, and urinary folate excretion. The subjects had the CC (normal), CT (heterozygote), or TT (homozygote) genotype for MTHFR. The subjects all showed moderate folate deficiency after the 7-week depletion phase and returned to folate sufficiency with 7 weeks of repletion with 400 µg DFE. There were no differences among plasma homocysteine levels. Guinotte et al. [13] stated that their data supported that the RDA of 400 µg DFE met the needs of these subjects.

Not all research shares these findings. In a study of 126 healthy subjects with different MTHFR genotypes, the C677T TT subjects had similar plasma folate, but higher plasma homocysteine levels than the CT or

CC subjects when consuming a folate-rich diet that contained an average of 660 DFEs [14]. These differences in plasma homocysteine levels disappeared when a supplement was added, boosting folate intake to an average of 814 DFEs. However, even with the consumption of 660 DFEs, the plasma homocysteine of the TT subjects was not above 12 µmol/L, a level that reflects folate sufficiency [14].

These findings can be compared to those of de Bree and coauthors [15] who studied data collected on 2,051 Dutch subjects and found that at a similar intake, subjects with the TT genotype for the C677T MTHFR had the lowest plasma folate and highest plasma homocysteine. However, these subjects were only consuming approximately 200 µg of folate per day, and the Dutch do not fortify grain products with folic acid [16].

The MTHFR polymorphisms appear to have their greatest impact on folate status when folate intake is low. The fortification policies of the United States and Canada ensure that folate intake has increased.

MTHFR Variations and Risk for Chronic Disease

Since the identification of the MTHFR polymorphisms, a great deal of research has investigated and reported risks for neural tube defects, cardiovascular disease, colorectal cancer, and dementias in people with poor folate status [1,17]. This research has an extra dimension when the MTHFR polymorphisms are also considered. This overview will limit its discussion of chronic disease risk to subjects with the C677T MTHFR low-activity (TT) genotype compared to those with the normal-activity (CC) genotype, since most research is about this variant.

Neural Tube Defects (NTDs) Bailey, Rampersaud, and Kauwell [16] reviewed the impact of folate fortification on improving folate status of Americans and Canadians, along with the decline in NTDs in both countries. While estimated to be between 15%–30% in the United States and greater in Canada, this decline may be due to several explanations in addition to positive effect of fortification. These explanations include more prenatal screening for NTDs, more research about the nutritional risk factors for NTDs, and an increased use of folic acid supplementation due to public education programs [18].

Botto and Yang [12] reviewed the relationship between NTD risk and the C677T mutation in several studies and reported that there was not a significantly increased risk for a NTD. Recently a study of 175 American Caucasians with NTDs, who had the TT genotype for the C677T mutation, reported that there was a nonsignificant

relationship to the parents' genotype. While prenatal folate is credited for a reduction in NTDs, the relationship appears to be more complicated than it originally appeared [19].

Cardiovascular Disease While the increased risk for cardiovascular disease in individuals with hyperhomocysteinemia is well documented in case control studies, some large prospective trials have not found the same relationship. Some argue that increases in plasma homocysteine are the outcome of renal atherosclerosis and decreased homocysteine excretion, not the cause of cardiovascular disease. This argument is reinforced by the general findings that individuals with the C677T TT genotype have higher plasma homocysteine levels, but do not have a greater risk of cardiovascular disease [20]. Others believe that the relationship of the TT genotype cannot be evaluated since the studies are too small to establish causality [20]. A recently published meta-analysis of 40 studies stated that individuals with the C677T TT genotype had a 16% higher odds ratio to cardiovascular disease compared to the CC genotype [21]. This was particularly true when the subjects had low folate status. Low dietary folate intake may be the explanatory factor in the manifestation of the effect of the MTHFR polymorphism on cardiovascular disease risk.

Dementias If homocysteine accumulates inside cells, the cells will evict this metabolic oxidant into the bloodstream. Homocysteine crosses the blood-brain barrier and is theorized to be neurotoxic [22]. Since research indicates that high plasma homocysteine is a risk factor for dementias and other cognitive problems (depression, psychosis), it logically follows that individuals with MTHFR genetic polymorphisms are at a greater risk. However, this relationship has not been verified [17,22]. A recent Cochrane review concluded that there was no effect of folic acid supplementation, with or without vitamin B₁₂, in treating mild or moderate problems with cognition, dementia or Alzheimer's disease [23].

Colorectal Cancer Poor folate status is positively correlated to a risk for colorectal cancer. This can be theoretically explained because a decrease in folate leads to DNA hypomethylation and increased uracil (instead of thymine) incorporation into DNA. The presence of uracil increases the activity of DNA repair mechanisms, but the repair may not be totally effective. Thus, DNA is more prone to damage, causing an increased risk to invasion by a cancer virus. The combination of DNA hypomethylation and increased uracil incorporation, caused by poor folate status,

explains the folate-cancer relationship [11]. Unlike other risks for disease that have been discussed, the MTHFR polymorphism may protect individuals from colorectal cancer. While studies generally report no difference in risk for the heterozygous (CT) or homozygous (TT), a trend in some studies indicates that as long as folate intake is high and alcohol intake is low, the TT homozygote may have a lower risk. This may be due to the shunting of N⁵N¹⁰-methylene tetrahydrofolate toward the formation of nucleotides (since MTHFR activity is low). Consequently, malformed DNA would not occur [11]. This possible protective relationship of the MTHFR polymorphism is preliminary, and more research is needed.

Summary

As with most scientific research, a variety of research and opinions surround the topic about the health implications for individuals having an MTHFR polymorphism. Some ethnic groups have a greater susceptibility to this condition, and it causes an increase in dietary folate needs. But it is not clear, once those dietary needs are met, if the individual has an increased risk for NTDs, cardiovascular disease, and dementias. The interaction of the genotype, dietary intake, other genetic factors, and the environment may explain the relationship.

This brief discussion about the possible effect of MTHFR polymorphisms and the impact on nutrigenomics currently has limited application to the general public for two reasons. First, there is currently little individualized genetic mapping. Second, the impact of having an MTHFR polymorphism on health is not clear. Both of these current limitations are likely to decline with enhanced technology and continued research. Future nutrition professionals may consider MTHFR, along with many other genetic traits, in their nutrition assessment and subsequent recommendations. Understanding how slight alterations in the human genome might affect nutrient needs and disease risk sheds light into the future of nutrition care.

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10

The Fat-Soluble Vitamins

Vitamin A and Carotenoids

Vitamin D

Vitamin E

Vitamin K

For each vitamin, the following subtopics (when applicable) are discussed:

Sources

Digestion and Absorption

Transport, Metabolism, and Storage

Functions and Mechanisms of Action

Interactions with Other Nutrients

Metabolism and Excretion

Recommended Dietary Allowance or Adequate Intake

Deficiency

Toxicity

Assessment of Nutriture

PERSPECTIVE

The Antioxidant Nutrients, Reactive Species, and Disease

This chapter addresses each of the four fat-soluble vitamins—A, D, E, and K—and the carotenoids. The reader is referred to Chapter 9 for an overview of vitamins and information pertaining to the water-soluble vitamins. The absorption and transport of the fat-soluble vitamins, in contrast to those of the water-soluble vitamins, are closely associated with the absorption and transport of lipids. As with dietary lipids, optimal fat-soluble vitamin absorption requires the presence of bile salts. Similarly, fat-soluble vitamins in the body initially are transported by chylomicrons. Moreover, the fat-soluble vitamins are stored in body lipids, although the amount stored varies widely among the four fat-soluble vitamins. Table 10.1 provides an overview of the discovery, function, deficiency syndrome, food sources, and recommended dietary allowance (RDA) or adequate intake (AI) of each of the fat-soluble vitamins. The RDAs and AIs for all nutrients and for all age groups are provided on the inside cover of the book.

Vitamin A and Carotenoids

The term *vitamin A* is generally used to refer to a group of compounds that possess the biological activity of all-*trans* retinol. The retinoids are structurally similar and include retinol, retinal, retinoic acid, and retinyl ester, as well as synthetic analogues. Structurally, retinoids contain a β-ionone ring and a polyunsaturated side chain, with either an alcohol group (retinol, Figure 10.1a), an aldehyde group (retinal, Figure 10.1b), a carboxylic acid group (retinoic acid, Figure 10.1c), or an ester group (retinyl ester such as retinyl stearate or palmitate, Figure 10.1d). The side chain is made up of four isoprenoid units with a series of conjugated double bonds. The double bonds may exist in a *trans* or a *cis* configuration.

Vitamin A was initially found to be an essential growth factor in animal foods and was called fat-soluble A. McCollum and Davis followed by Osborne and Mendel are credited in about 1915 with its discovery.

Provitamin A carotenoids represent a group of compounds that are precursors of vitamin A. Although more than 600 carotenoids (lipid-soluble red, orange, and yellow pigments produced by plants) exist, fewer than 10% are thought to exhibit vitamin A activity. In other words, fewer than 60 can be converted to retinol. Structurally, carotenoids have an expanded carbon chain containing conjugated double bonds usually, but not always, with an unsubstituted β-ionone ring at one or both ends of the chain. Three dietary provitamin A carotenoids, which are found most often in the all-*trans* form but can occur as *cis* isomers, are β-carotene (Figure 10.1e), α-carotene (Figure 10.1f), and

Table 10.1 The Fat-Soluble Vitamins: Discovery, Function, Deficiency Syndrome, Food Sources, and Recommended Dietary Allowance (RDA) or Adequate Intake (AI)

Vitamin	Discovery	Biochemical or Physiological Function	Deficiency Syndrome or Symptoms	Good Sources in Rank Order	RDA or AI
Vitamin A (retinol, retinal, retinoic acid)	McCollum (1915)	Synthesis of rhodopsin and other light receptor pigments; metabolites involved in growth, cell differentiation, bone development, and immune function	Poor dark adaptation, night blindness, xerosis, keratomalacia, xeroderma, Bitot's spots	Beef liver, dairy products, sweet potato, carrots, spinach, butternut squash, greens, broccoli, cantaloupe	900 µg RAE ^a 700 µg RAE ^b
Provitamins Carotenoids, particularly β-carotene					
Vitamin D	McCollum (1922)	Regulator of bone mineral metabolism, blood calcium homeostasis, and cell differentiation, proliferation, and growth	Children: rickets Adults: osteomalacia	Synthesized in skin exposed to ultraviolet light; fortified milk	5–15 µg ^{c,d,e}
Provitamins Ergosterol 7-dehydrocholesterol					
Vitamin D ₂ (ergocalciferol)					
Vitamin D ₃ (cholecalciferol)					
Vitamin E Tocopherols Tocotrienols	Evans and Bishop (1922)	Antioxidant	Infants: anemia Children and adults: neuropathy and myopathy	Vegetable seed oils	15 mg α-tocopherol ^c
Vitamin K	Dam (1935)	Activates blood-clotting factors II, VII, IX, X by γ-carboxylating glutamic acid residues; carboxylates bone and kidney proteins	Children: hemorrhagic disease of newborns Adults: defective blood clotting	Synthesized by intestinal bacteria; green leafy vegetables, soy beans, beef liver	120 µg ^{a,e} 90 µg ^{b,e}
Phylloquinones Menaquinones Menadione					

^aAdult males.^bAdult females.^cBoth males and females.^dVaries with age for adults, see text.^eAdequate intake.

β-cryptoxanthin (Figure 10.1g). Although not all carotenoids are vitamin A precursors, many carotenoids, such as lycopene (an open-chain analog of β-carotene; Figure 10.1h), and many oxycarotenoids (also called oxygenated carotenoids), such as canthaxanthin (Figure 10.1i), lutein (Figure 10.1j), and zeaxanthin, are thought to be of physiological importance to the body.

SOURCES

Both retinoids (often called preformed vitamin A or simply vitamin A) and carotenoids are found naturally in foods. Vitamin A is found primarily in selected foods of animal origin, especially liver and dairy products (including whole milk, cheese, and butter) as well as fish such as tuna, sardines, and herring. Some products, such as margarine, also may be fortified with vitamin A. Liver oils of fish (such as cod liver oil) are also high in vitamin A. The main form of vitamin A in foods is as retinyl esters such as retinyl palmitate, shown in Figure 10.1k. Retinoids are lipid soluble and can undergo oxidation if exposed to varying degrees of, for example, oxygen, light, heat, and some metals. In pharmaceutical vitamin preparations, all-trans retinyl acetate and all-trans retinyl palmitate are commonly used. Aquasol A,

a water-miscible form of the vitamin, is available for people with a fat malabsorptive disorder.

Carotenoids are synthesized by a wide variety of plants and thus are found naturally in many fruits and vegetables. One of the most abundant carotenoids is β-carotene, which exhibits the greatest amount of provitamin A activity. Other common dietary carotenoids include α-carotene and β-cryptoxanthin (both provitamin A carotenoids) along with lycopene, lutein, and zeaxanthin. In general, yellow, orange, and red (brightly colored) fruits and vegetables such as carrots, watermelon, papayas, tomatoes, tomato products (ketchup, chili sauce, spaghetti sauce), squash, pink grapefruit, and pumpkins provide significant amounts of carotenoids. Green vegetables also contain some carotenoids, although the pigment cannot be seen because it is masked by chlorophyll. Carrots typically represent a major source of both α- and β-carotene in American diets. Other major dietary contributors of β-carotene include broccoli, cantaloupe, squash, peas, and spinach. Fruits provide much of the dietary β-cryptoxanthin, and tomatoes, along with tomato sauces and watermelon, are good sources of dietary lycopene, a carotenoid that is red in color. Good sources of zeaxanthin include peppers (orange), corn, potatoes, and eggs. Broccoli, beets,

kiwi fruit, and eggs provide some lutein. Canthaxanthin, a red-orange carotenoid, is found in plants as well as in fish and seafood such as sea trout and crustaceans. Meat and fish are not major sources of carotenoids, but because animals and fish feed on plants, they can accumulate some carotenoids. Carotenoids also may be added to foods. β -carotene and canthaxanthin, for example, are approved by the Food and Drug Administration for use as a food color additive.

DIGESTION AND ABSORPTION

Vitamin A, because it is bound to other food components, requires some digestion before it can be absorbed into the body. Retinol, for example, is typically bound to fatty acid esters, the most common of which is retinyl palmitate

(Figure 10.1k). Furthermore, retinyl esters and carotenoids in foods are often complexed with protein from which they must be released. Although heating plant foods weakens some complexes, such as protein-carotenoid complexes, enzymatic digestion is still required. Carotenoids and retinyl esters from protein (as shown in Figure 10.2) initially are hydrolyzed by the action of pepsin in the stomach. Because of their fat solubility, the freed (i.e., no longer bound to protein) retinyl esters and carotenoids typically coalesce to form fat globules in the stomach. These fat globules containing the vitamin are emptied into the duodenum. Proteolytic enzymes in the duodenum also can hydrolyze any protein-bound retinyl esters or carotenoids not freed in the stomach. Hydrolysis of retinyl and carotenoid esters by various hydrolases and esterases occurs at the same

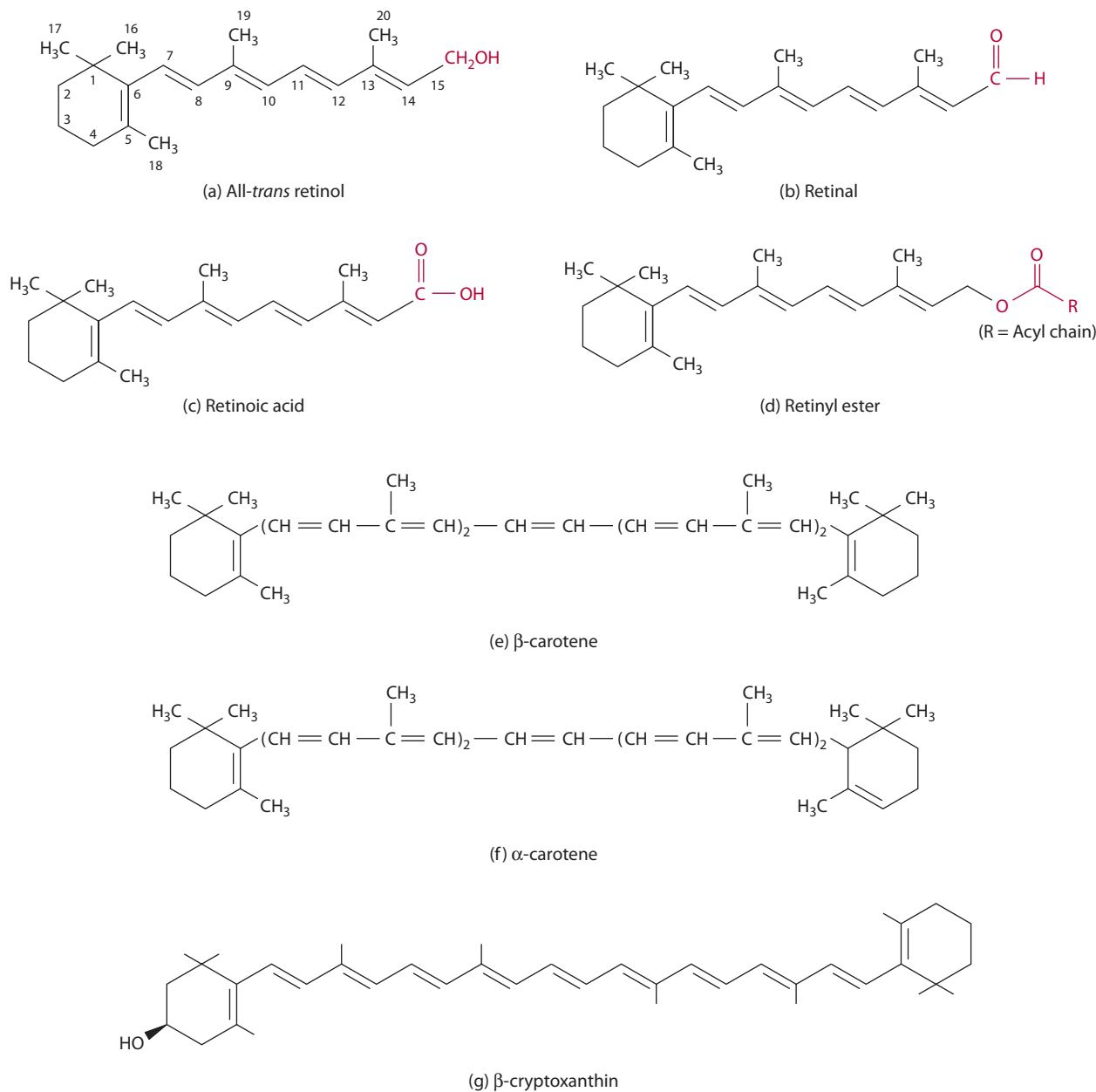
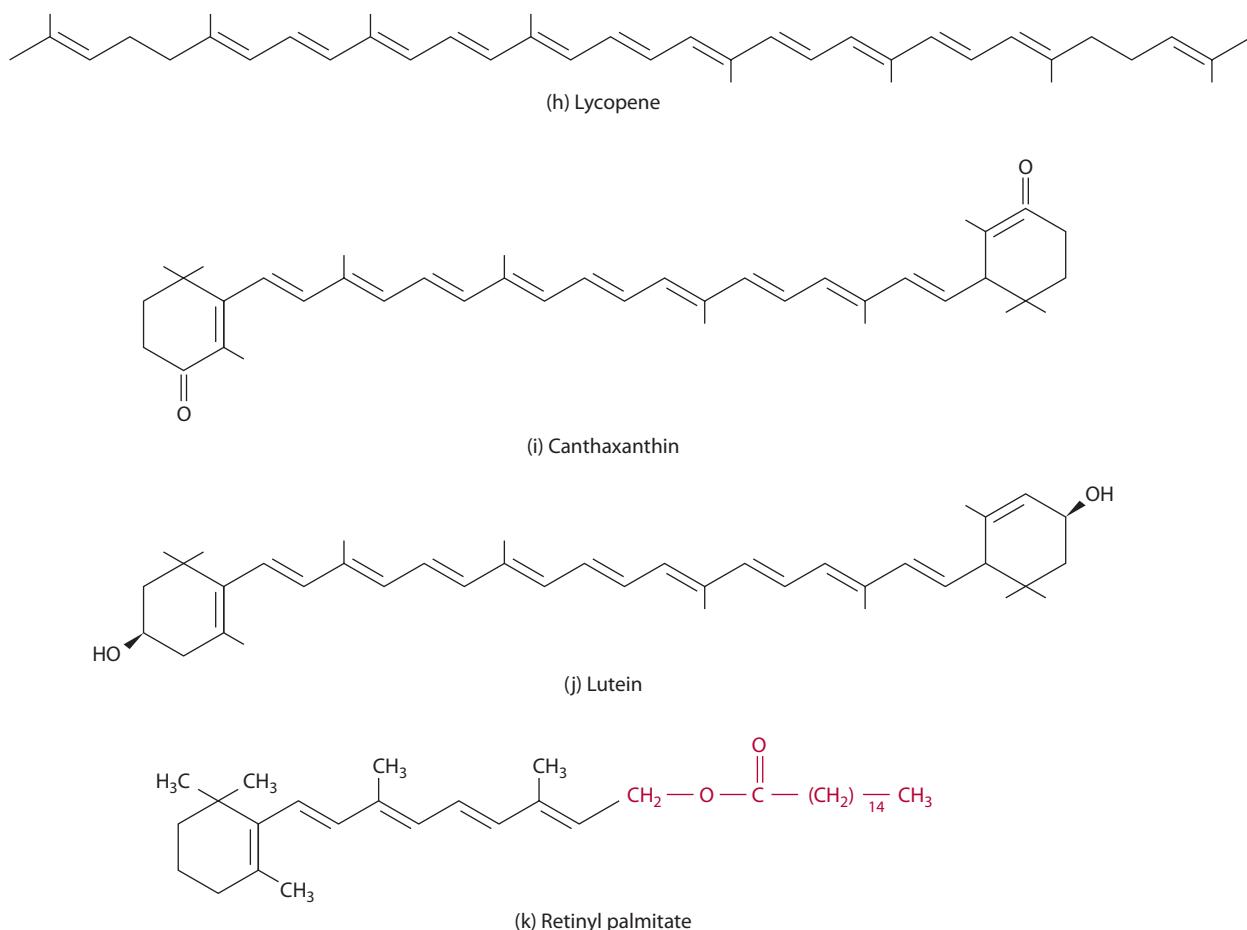


Figure 10.1 Vitamin A and carotenoid structures (continued on next page).

**Figure 10.1** (continued) Vitamin A and carotenoid structures.

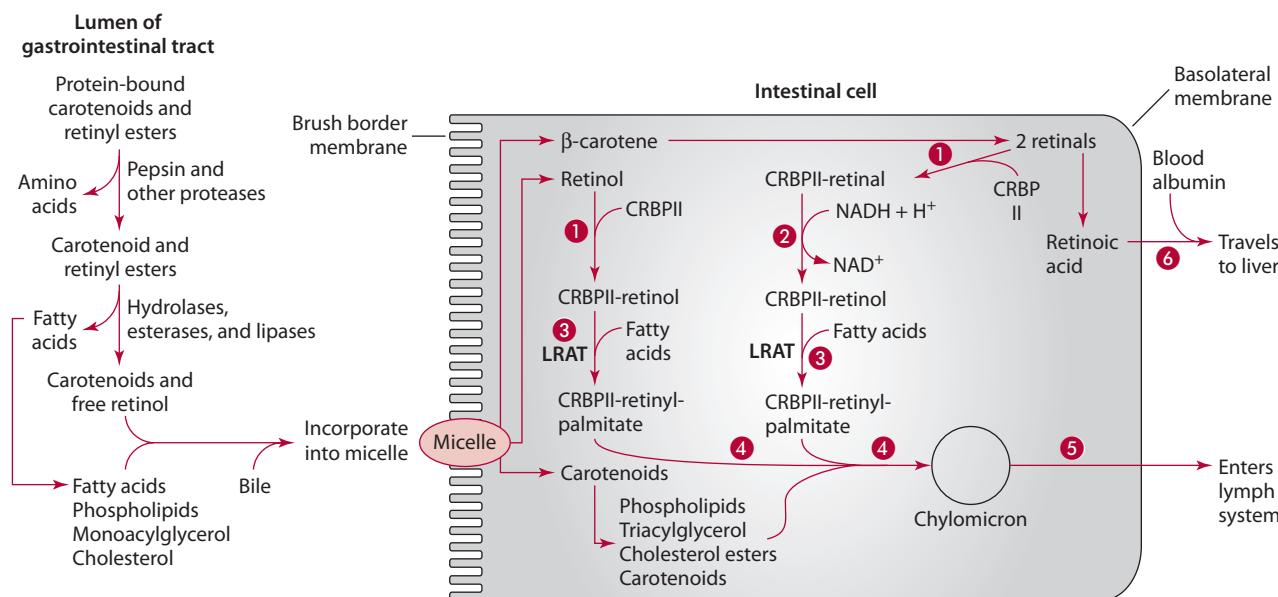
time that triacylglycerols, phospholipids, and cholestereryl esters are being hydrolyzed by pancreatic enzymes. Pancreatic lipase and pancreatic cholesterol ester hydrolase are secreted into the lumen of the small intestine to facilitate lipid and vitamin A digestion, and enzymes such as retinyl ester hydrolase also function on the intestinal brush border to digest the vitamin. Pancreatic hydrolases cleave shorter-chain retinyl esters, whereas intestinal brush border hydrolases act on longer-chain retinyl esters. Bile is also important to emulsify the fat globules containing the vitamin and other fats (emulsification results in large fat globules being broken up into smaller droplets).

Micelles form within the lumen of the small intestine from bile salts, phospholipids, monoacylglycerol, and retinyl and carotenoid esters. The released or now free carotenoids and retinols in the small intestine remain solubilized in micellar solutions along with the other fat-soluble food components. The micellar solutions containing the carotenoids and preformed vitamin A are absorbed across the microvilli brush border membrane of the duodenum and jejunum and into the enterocyte. When retinol is ingested in physiological amounts, absorption is thought to occur by a specific protein carrier in the brush border of the enterocyte [1]. Absorption of preformed vitamin A following ingestion

of pharmacological doses of the vitamin is thought to be nonsaturable [1]. Carotenoids also are absorbed by both carotenoid transporters and passive diffusion [2].

The efficiency of absorption differs between preformed vitamin A and carotenoids. Approximately 70% to 90% of dietary preformed vitamin A is absorbed as long as the meal contains some (~10 g or more) fat [3]. Dietary carotenoid absorption varies considerably, depending on food processing and typically is less than that of the retinoids. Carotenoid absorption ranges from about <5% for carotenoids from uncooked vegetables or non-heat processed vegetable juices to about 60% if present as a pure oil or as part of an aqueous dispersion supplement [3–5]. Fiber (especially pectin) intake as well as excessive vitamin E consumption can diminish carotenoid absorption. Pectin appears to diminish absorption by interfering with micelle formation. In addition, various carotenoids appear to interact to influence (enhance or inhibit) individual carotenoid absorption. Figure 10.2 depicts the digestion and absorption of preformed vitamin A and β -carotene.

Within the intestinal mucosal cell/enterocyte (and to some extent in the liver, adipose tissue, and lungs, among other organs), some carotenoids, including α - and β -carotene and cryptoxanthin, undergo metabolism. The extent



- ① Cellular retinol-binding protein (CRBP) II binds to both retinol and retinal in the intestinal cell.
- ② Retinal, while attached to CRBPII, is converted to retinol to form CRBPII retinol.
- ③ Lecithin retinol acyl transferase (LRAT) esterifies a fatty acid (palmitic acid) onto the CRBPII-bound retinol to form CRBPII-retinylpalmitate.
- ④ Retinyl esters are incorporated along with phospholipids, triacylglycerol, cholesterol esters, carotenoids, and apoproteins to form a chylomicron.
- ⑤ Chylomicrons leave the intestinal cell and enter the lymph system and ultimately the blood.
- ⑥ Retinoic acid can directly enter the blood where it attaches to albumin for transport to the liver.

Figure 10.2 Digestion and absorption of carotenoids and vitamin A, and reesterification of retinol in the intestinal cell.

to which provitamin A carotenoids are converted to retinoids is influenced by several factors, such as the vitamin A status of the person and the amounts and forms of the carotenoids consumed. Within the enterocyte, for example, β -carotene is hydrolyzed by noncentral cleavage or by β -carotene 15,15'-oxygenase (this oxygenase is also found in the liver, lungs, kidney, and retina). Other enzymes involved in carotenoid metabolism also have been identified in various species, as reviewed by Yeum and Russell [6]. Noncentral cleavage generates several metabolites (alcohols, aldehydes, etc.) [7], whereas oxygenase activity converts one molecule of β -carotene into two molecules of retinal (also called retinaldehyde) (Figure 10.3). However, because the activity of β -carotene 15,15'-oxygenase is relatively low, only about 60% to 75% of the β -carotene is hydrolyzed [8]. The presence of vitamin E is thought to protect β -carotene and its product from oxidation; large doses of vitamin E, however, may inhibit β -carotene absorption or its conversion to retinol in the intestine [3]. Up to about 15% of β -carotene may leave the intestine intact (i.e., without oxidation) for transport by chylomicrons to tissues. An estimated 12 μg β -carotene or 24 μg α -carotene or β -cryptoxanthin is required to produce the vitamin A activity of 1 μg retinol.

In addition to intestinal cell carotenoid metabolism, retinoid metabolism also occurs as shown in Figure 10.4.

Retinal, for example, can be converted to retinol by retinal reductase (also called retinal oxidase), an NADH/NADPH-dependent enzyme. Some retinol that is generated may be then converted to retinyl β -glucuronide. In addition, some of the retinal may be irreversibly oxidized to retinoic acid within the intestinal cell. Some of the retinoic acid may be conjugated to form retinoyl β -glucuronide (RAG). RAG and retinoic acid, in contrast to retinol, can enter circulation through the portal vein. Retinoic acid is transported in the plasma bound tightly to albumin (Figure 10.2). RAG concentrations in the plasma are typically low, but RAG appears to function in part like retinoic acid in tissues. RAG promotes growth and cell differentiation, but it does not bind to nuclear retinoic acid receptors.

To leave the intestinal cell and travel to other tissues in the body, retinol must be esterified and incorporated into a chylomicron. Retinol formed from retinal that was generated from the oxidation of carotenoids follows the same metabolic pathways of reesterification in the intestinal cell as does retinol originating from dietary retinyl esters. One of two metabolic pathways may be followed for retinol reesterification in the enterocyte. The primary pathway involves cellular retinol-binding protein (CRBP) II. CRBPs, part of a group of low-molecular-weight lipid-binding proteins, are thought to help regulate retinol

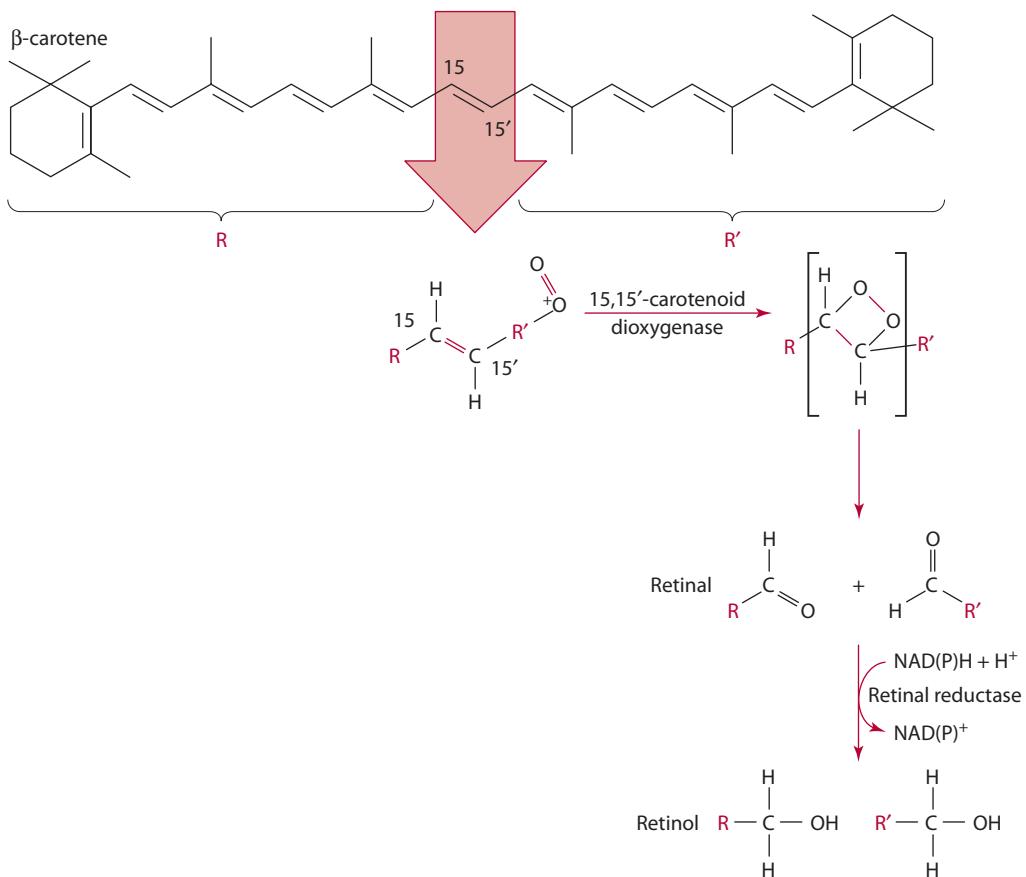


Figure 10.3 Cleavage of carotene to retinal and its reduction to retinol.

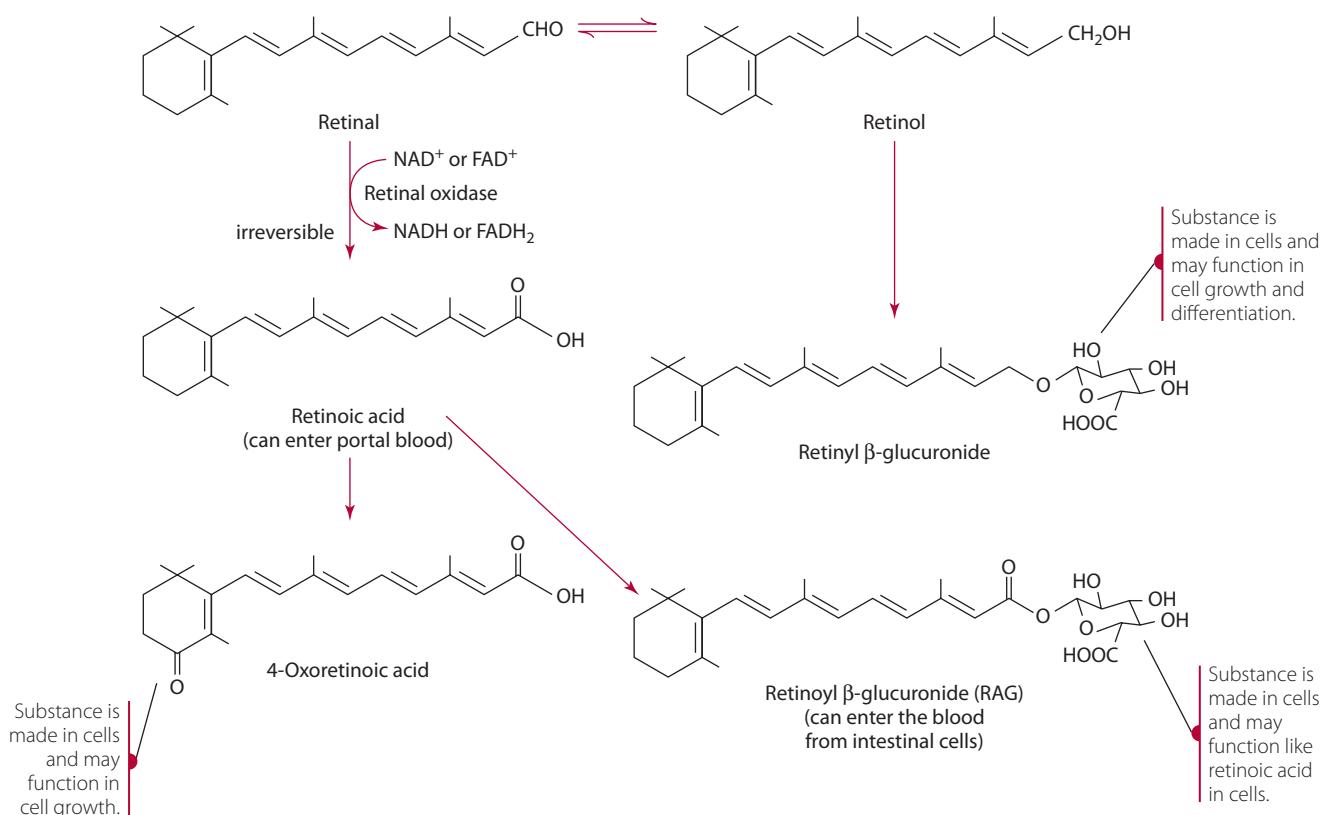


Figure 10.4 Retinal metabolism.

use in cells. CRBPII binds both retinol and retinal and is present in the cytoplasm of epithelial cells of the small intestine [1]. CRBPII directs the reduction of retinal and subsequent esterification. CRBPII-bound retinol is esterified by lecithin retinol acyl transferase (LRAT) to form mainly retinyl palmitate, but also retinyl stearate and retinyl oleate, among others. LRAT specifically transfers sn-1 fatty acids from membrane-associated phosphatidylcholine to retinol that is bound to CRBPs. LRAT is thought to be the main enzyme responsible for esterification in the small intestine, liver, retinyl pigment epithelium, and likely other tissues [9]. The enzyme appears to be upregulated by all-*trans* retinoic acid and synthetic retinoids that bind to the RAR family of nuclear receptors and ultimately induce LRAT gene expression [9] (see page 383, gene expression). The minor second pathway for reesterification involves binding of retinol to a cellular protein that is nonspecific, with subsequent reesterification by acyl CoA retinol acyl transferase (ARAT). ARAT may serve to esterify retinol when large doses of the vitamin are ingested [10].

TRANSPORT, METABOLISM, AND STORAGE

Within the intestinal cell, the newly formed retinyl esters, along with a small amount of unesterified retinol and any carotenoids that have been absorbed unchanged, are incorporated into chylomicrons containing cholesterol esters, phospholipid, triacylglycerols, and apoproteins. These chylomicrons are then carried first into the lymphatic system and then into general circulation (i.e., the blood). Chylomicrons deliver retinyl esters, some unesterified retinol, and carotenoids to many extrahepatic tissues, including bone marrow, blood cells, spleen, adipose tissue, muscle, lungs, and kidneys. Retinyl esters and carotenoids not taken up by peripheral tissue are transported to the liver as part of the chylomicron remnant. About 70% to 75% of chylomicron retinoids are cleared from circulation by the liver [11].

Carotenoids and vitamin A reaching the liver typically undergo additional metabolism. For example, carotenoids reaching the liver can follow three routes: cleavage to form retinol, incorporation into and release as part of very low density lipoproteins (VLDLs) or other lipoproteins for transport to other tissues, and storage in the liver (or adipose tissue).

The handling of retinyl esters that reach the liver is shown in Figure 10.5. However, most cells of the body are able to metabolize retinol generated from the retinyl esters through a number of metabolic pathways. The retinyl esters are hydrolyzed by a retinyl ester hydrolase following their uptake by the hepatic **parenchymal cells** (functional cells of an organ). Within the cell, retinol binds with a cellular retinol-binding protein (CRBP). CRBPs have been found in many body cells. CRBPI is present in all tissues, but is found in especially high concentrations in the liver

and kidney. CRBPII is found in especially high amounts in the intestine, especially the jejunum. CRBPIII is present in relatively high concentrations in the liver, skeletal muscle, kidney, and heart; CRBPIV is mostly found in the heart, kidney, and sections of the colon. CRBP is thought to function both to help control concentrations of free retinol within the cell cytoplasm and thus prevent its oxidation and to direct the vitamin, through a series of protein-protein interactions, to specific enzymes of metabolism [12]. CRBP may also assist in the transfer of retinal across organelles for metabolism. Enzymatic metabolism of retinol as shown in Figure 10.5 includes possible esterification by enzymes such as LRAT, if retinol is bound to CRBP, or ARAT, if retinol is unbound. Unbound CRBP concentrations are thought to inhibit LRAT and thereby prevent esterification for storage. CRBP-bound retinol also may be oxidized to retinal by NAD(P)H-dependent retinol dehydrogenase or phosphorylated to retinyl phosphate by ATP for glycoprotein functions [12].

Retinol that has been esterified may be stored in the liver. Some storage of retinol occurs in the parenchymal cells, but about 80% to 95% of the retinol is stored in the liver in small perisinusoidal cells called **stellate cells** (also known as Ito cells). Note that retinoic acid does not accumulate in appreciable amounts in the liver or other tissues. In the stellate cells, vitamin A (retinol) is stored as retinyl esters (primarily retinyl palmitate, but also retinyl stearate, oleate, and linoleate) with lipid droplets. Hydrolases can release the retinol from its stores as needed for use. With adequate liver stores of vitamin A (minimum 20 µg vitamin A per g liver) [3], plasma vitamin A concentrations remain fairly constant over a wide range of dietary intakes. Only after the hepatic stellate cells can accept no more retinol for storage does hypervitaminosis A occur (see p. 389).

Retinol transport in the blood requires two specific proteins, retinol-binding protein (RBP) and transthyretin (TTR), formerly known as prealbumin and as thyroxine-binding globulin. Both proteins are synthesized by hepatic parenchymal cells. Initially, for hepatic release into the blood, each mol of retinol released by a hydrolase from its ester storage form combines with 1 mol of RBP to form holo-RBP (Figure 10.5). Synthesis of RBP is dependent on a person's protein, retinol, and zinc status. Holo-RBP (which contains the vitamin A bound in an interior portion or hydrophobic region of the complex) then interacts with 1 mol of TTR, a tetrameric protein (Figure 10.5). In the plasma, the holo-RBP-TTR complex circulates bound to thyroxine (T_4) as part of a trimolecular complex (Figure 10.5), with a half-life of about 11 to 15 hours. Blood concentrations of the complex remain fairly consistent unless the person chronically consumes inadequate vitamin A. Normal plasma retinol concentrations range from about 1.05 to 3 micromol/L (30 to 86 µg/dL) and remain fairly constant even when total hepatic retinol concentrations vary greater than 15-fold [9].

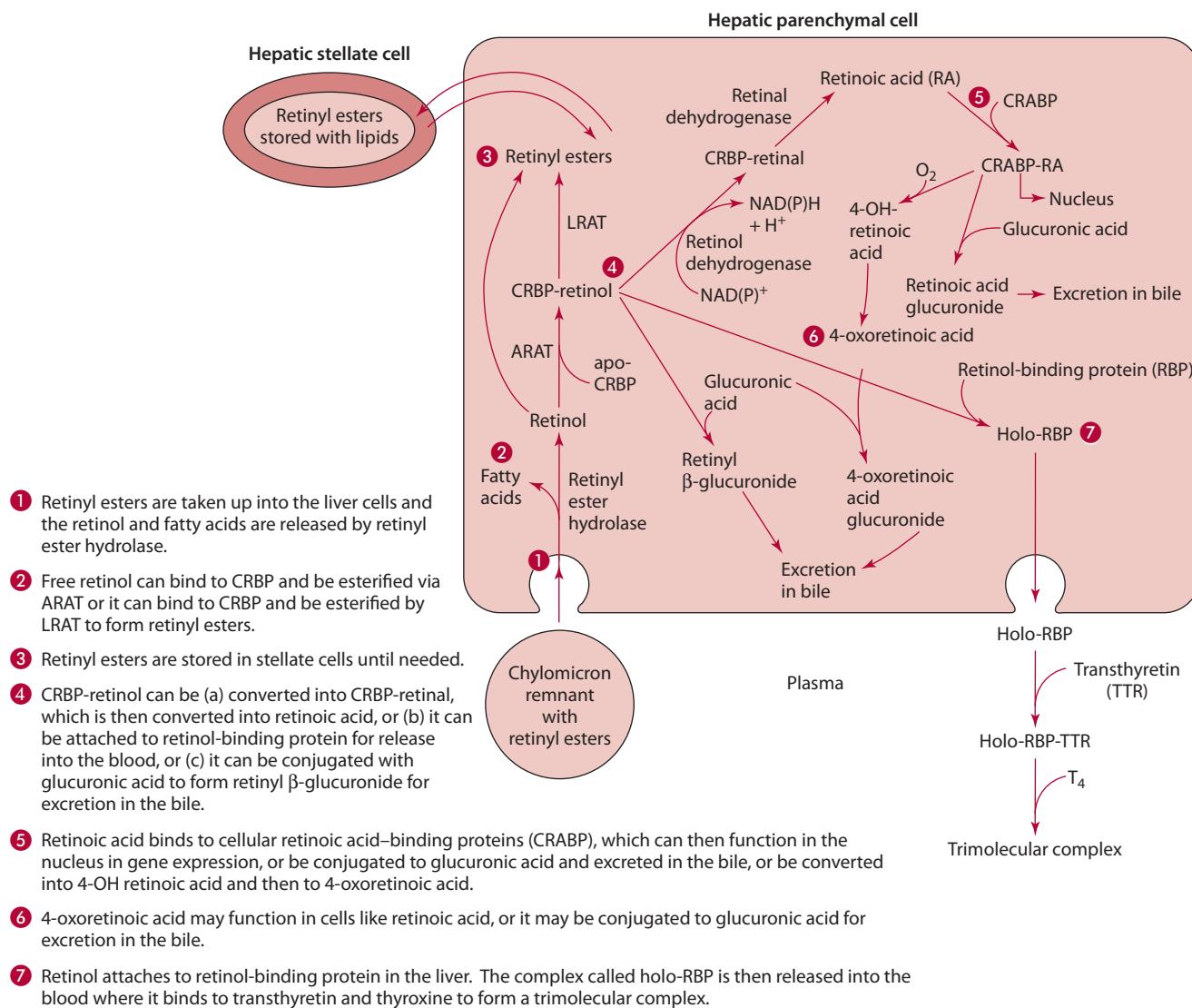


Figure 10.5 Vitamin A metabolism in the liver.

Uptake of retinol from the complex by tissues is not completely understood, but it is thought to be mediated by cellular RBP receptors and by a receptor-independent mode depending on the specific tissue [13–15]. Factors influencing uptake are not known but may involve CRBP concentrations or saturation, and the extent of intracellular retinol metabolism, among others. For receptor-dependent uptake, TTR is thought to dissociate from the complex, whereas holo-RBP binds to the cellular RBP receptor. The complex is then endocytosed with cytosolic release of vitamin A and apo-RBP. Apo-RBP is thought to be secreted back into the blood for reuse or degradation by the kidney. Some of the many tissues that use vitamin A from the complex include adipose tissue, skeletal muscle, lungs, kidneys, eyes, white blood cells, and bone marrow. Retinol is extensively recycled among plasma, extrahepatic tissues, and the liver before degradation [9,10].

Carotenoids are also found in the blood and are transported as part of lipoproteins. Carotenoids such as

β-carotene and lycopene are thought to concentrate in the hydrophobic core of lipoproteins for serum transport, whereas carotenoids with polar groups are found partly on the lipoprotein surface [5,16]. β-carotene, α-carotene, and lycopene distribution among lipoproteins is similar: low-density lipoproteins (LDLs) carry 58% to 73%, high-density lipoproteins (HDLs) carry 17% to 26%, and very low density lipoproteins (VLDLs) carry 10% to 16% [16]. In contrast, lutein and zeaxanthin (polar carotenoids) are carried predominantly (53%) by HDLs, but also by LDLs (31%) and VLDLs (16%) in a fasting state [16]. Serum carotene concentrations reflect recent intake and not body stores. The most common serum carotenoids are β-carotene, α-carotene, lycopene, lutein, zeaxanthin, and cryptoxanthin.

Uptake of carotenoids into target tissues differs from that of retinol. Carotenoids are taken up as part of the lipoprotein, with uptake mediated by specific apoprotein receptors found in a variety of tissues. Carotenoids are stored mainly

in the liver and adipose tissue, but some specific tissues concentrate specific carotenoids. For example, the retina of the eye is relatively rich in lutein and zeaxanthin.

In contrast to retinol, which is mobilized from the liver for transport to other tissues, retinoic acid is thought to be produced in small amounts by individual cells. Whether retinoic acid is centrally produced by the intestine or the liver for transport to other tissues is unclear. Retinoic acid is usually bound to albumin for transport in the blood, but plasma retinoic acid concentrations typically are low. Within the cell cytoplasm, retinoic acid binds to cellular retinoic acid–binding proteins (CRABPs). CRABPs are thought to function in a capacity similar to that described for CRBPs. Although both CRBPs and CRABPs are often found in the same tissues, their relative distribution in the tissues differs. CRABP, like CRBP, helps to solubilize retinoic acid, regulate metabolism of retinoic acid within the cell, and direct the usage of retinoic acid intracellularly. Cytochrome P₄₅₀RAI, also called CYP26, catalyzes the oxidation and glucuronidation of all-trans retinoic acid–generating polar metabolites. CYP26 (a subfamily of cytochrome P₄₅₀ enzymes) is found in the liver and brain among other tissues and its expression appears to be positively regulated by vitamin A [9].

FUNCTIONS AND MECHANISMS OF ACTION

Vitamin A

Vitamin A is recognized as being essential for vision as well as for cellular differentiation, growth, reproduction, bone development, and immune system actions. This section reviews each of these functions before addressing the functions of the carotenoids.

Vision Several parts of the eye work together to ensure vision. For example, light enters the eye through the cornea, the outermost tissue that covers the front of the eye. The muscles of the iris adjust the size of the pupil in response to the dimness/brightness of the light. The light then passes through the lens and the vitreous humor (which shapes the eye) and hits the retina, the inner lining at the back of the eye. The retina contains specialized cells, called rods and cones, which act as photo- or light receptors. Cones are found near the center of the retina and function especially during the day in bright light. As darkness falls/light dims, the rods serve as the photo- or light receptors. Vitamin A is needed to form **rhodopsin** (a vitamin A-containing pigment protein) found in the rods. Rhodopsin is made up of vitamin A as *cis*-retinal and the protein opsin (Figure 10.6).

In basic terms (Figure 10.6), in a dim/dark environment, when a flash of light hits the retina, rhodopsin is cleaved. As the rhodopsin is cleaved, opsin is released, the *cis*-retinal is converted to *trans*-retinal, and signals are sent to the part of the brain involved with eyesight. To regain vision in the dark, the rhodopsin molecule must be remade using

vitamin A as *cis*-retinal. The *trans*-retinal that is released is transported from the photoreceptor rod cells into the pigment epithelium of the retina, where it is converted back to *cis*-retinal. The *cis*-retinal is then transported back to the rod cell where it reattaches to opsin to form rhodopsin. A failure or delayed recovery of vision in the dark following a light flash is called night blindness, and may be caused by inadequate vitamin A.

Figure 10.7 shows the visual cycle (the formation and reformation of rhodopsin after its degradation) in more detail. This section focuses on how vitamin A is metabolized as part of the visual cycle. Retinol is transported to the retina by the blood as part of the holo-RBP-TTR complex. Retinol next moves into the pigment epithelium of photoreceptor rod cells (Figures 10.7 and 10.8). Within the pigment epithelium, much of the retinol is converted by LRAT to all-*trans* retinyl esters and some into 11-*cis* retinyl esters. The all-*trans* retinyl esters in turn may be stored in the pigment epithelium and metabolized, by an all-*trans* retinyl ester isomeroxydrolase, as needed, to generate 11-*cis* retinol plus a fatty acid. Similarly, the 11-*cis* retinyl esters may be hydrolyzed, as needed, to 11-*cis* retinol and a fatty acid by retinyl ester hydrolase.

For the visual cycle to operate effectively, conversion of 11-*cis* retinol to 11-*cis* retinal is necessary. The 11-*cis* retinal must then be transported into the photoreceptor rod cells. The synthesis of 11-*cis* retinal occurs within the pigment epithelium by the action of 11-*cis* retinol dehydrogenase, which uses either NAD⁺ or NADP⁺. Transport of 11-*cis* retinol and 11-*cis* retinal is accomplished in the pigment epithelium by cellular retinal-binding protein (CRALBP), and transport of all-*trans* retinol and 11-*cis* retinal between the pigment epithelium and the photoreceptor rod cells (and thus across the interphotoreceptor matrix/space) is accomplished by interstitial or interphotoreceptor retinol-binding protein (IRBP). Specifically, IRBP, a glycolipoprotein, resides within the retinal interphotoreceptor space that lies between the pigment epithelium and the photoreceptor cells. CRBP and CRABP are also found in the retina.

Within the photoreceptor cells, 11-*cis* retinal binds as a protonated Schiff base to a lysine amino acid residue in the protein opsin (Figure 10.6) to produce the compound rhodopsin. Rhodopsin is embedded in disks located in the rod's outer segment, which is enclosed within a restricted compartment of the retina created by tight junctions between cells (Figure 10.8). The cells on the blood side are one layer thick and form the pigment epithelium. The “outer limiting membrane” is formed on the vitreal side of the photoreceptor cells by specific junctions between the photoreceptor cells and the Müller cells (Figure 10.8).

Within the photoreceptor cells, rhodopsin is able to detect small amounts of light, important for night vision. When a quantum of light (hv) hits the rhodopsin, changes occur in the vitamin A portion of the molecule such that 11-*cis* retinal is photoisomerized to generate all-*trans*

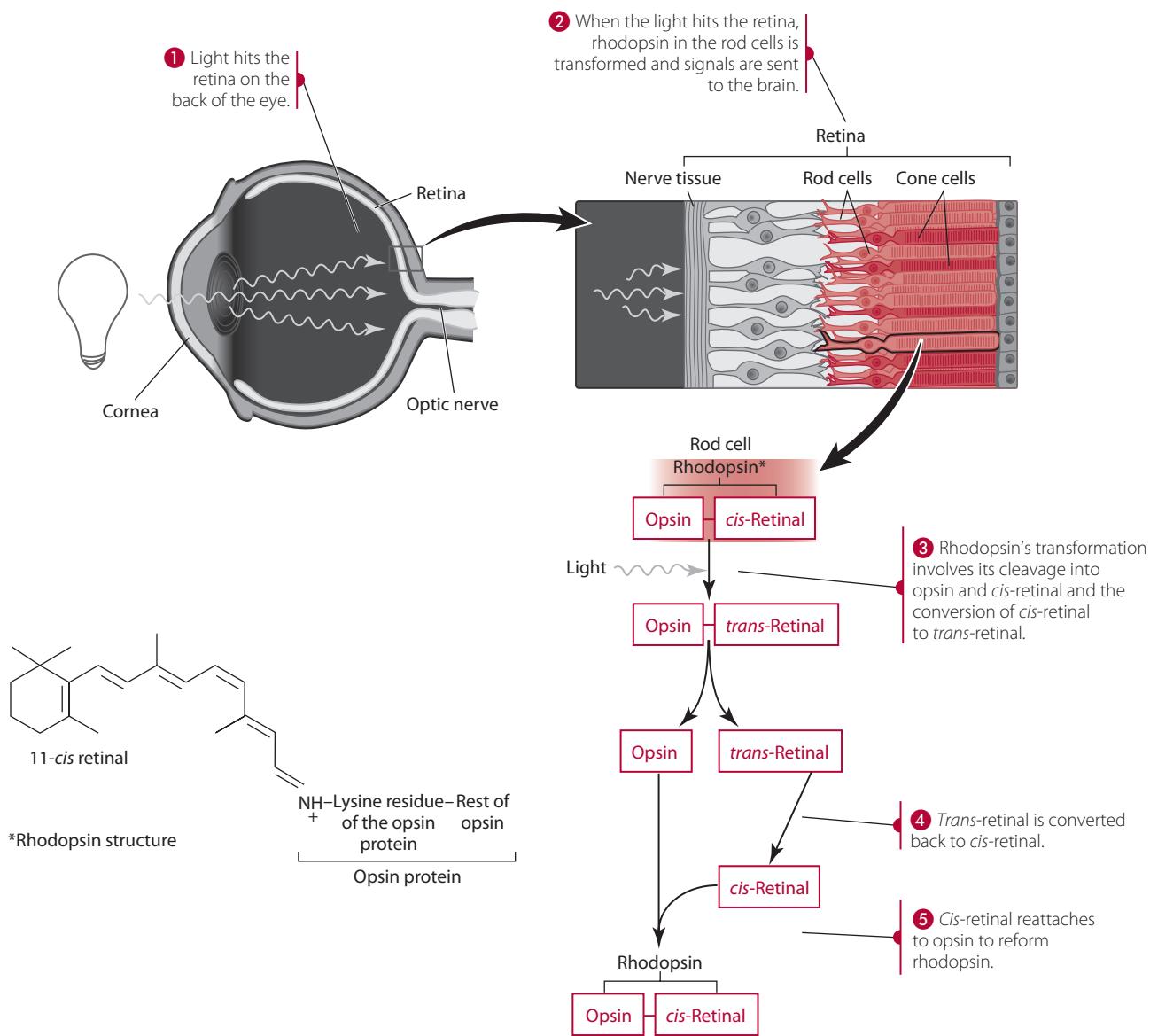


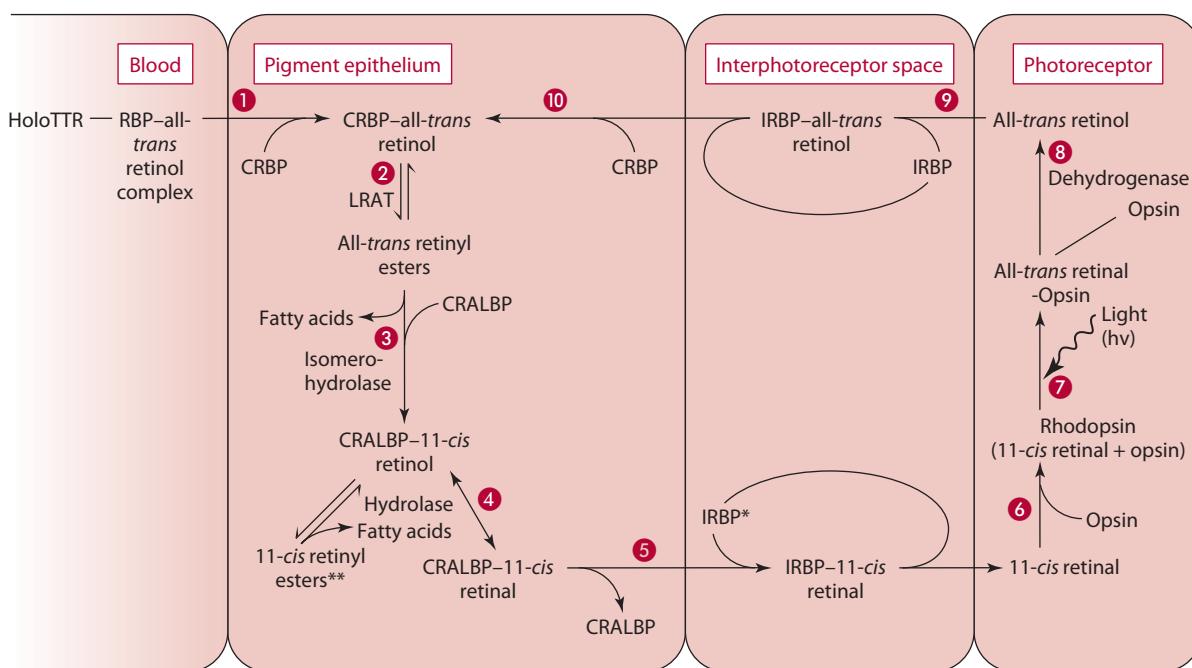
Figure 10.6 An overview of the role of vitamin A as a part of rhodopsin in vision.

retinal (Figures 10.6 and 10.7). The term *bleaching* is often used to describe this event, because a loss of color occurs. A chain of events triggered by the absorption of light by 11-*cis* retinal results in an electrical signal or impulse to and along the optic nerve to the brain. To transmit through the cell to the plasma membrane the message that light has hit the rhodopsin, a cascade of reactions thought to involve **transducin** (a G protein), phosphodiesterase, and cGMP occurs. This reaction cascade causes sodium channels in the plasma membrane to be blocked and the rod cell to hyperpolarize, resulting in signals by the optic nerve leading to specific areas of the brain.

The all-*trans* retinal formed as a result of light must be converted back to 11-*cis* retinal, and the rhodopsin must be regenerated. The steps for this conversion are thought to involve hydrolysis of all-*trans* retinal from the rhodopsin, reduction of all-*trans* retinal to all-*trans* retinol by an

NADPH-dependent retinol dehydrogenase, and transport of all-*trans* retinol across the interphotoreceptor matrix and into the retinal pigment epithelium by IRBP (Figure 10.7). Within the pigment epithelium, the all-*trans* retinol may be metabolized to all-*trans* retinyl esters, which may be directly isomerized to 11-*cis* retinal and subsequently oxidized to 11-*cis* retinal. Stored all-*trans* retinyl esters also may be mobilized from the pigment epithelium for isomerization and oxidation to yield 11-*cis* retinal. This compound may undergo transport back into the photoreceptor cells to bind again with opsin and complete the visual cycle.

Cellular Differentiation Vitamin A, especially as retinoic acid, is needed by many cells for cell differentiation. Cell differentiation is the process whereby an immature cell is transformed into a specific type of mature cell. Typically, for example, in rapidly dividing cells, the vitamin induces



* IRBP-Interphotoreceptor retinol-binding protein.

** Stored until needed.

- ① All-trans retinol moves out of the blood [where it is found as part of a complex with transthyretin (TTR) and retinol-binding protein (RBP)] and into the pigment epithelium in the rod cell. In the pigment epithelium it attaches to CRBP (cellular retinol binding protein).
- ② All-trans retinol is converted into all-trans retinyl esters by LRAT.
- ③ All-trans retinyl esters are converted to 11-cis retinol which is then attached to cellular retinal-binding protein (CRALBP).
- ④ 11-cis retinol is converted to 11-cis retinal while attached to CRALBP.
- ⑤ 11-cis retinal detaches from CRALBP and attaches to interphotoreceptor retinol-binding protein (IRBP) for transport across the interphotoreceptor space and into the photoreceptor. IRBP releases the 11-cis retinal upon delivery into the photoreceptor.
- ⑥ 11-cis retinal attaches to opsin to form rhodopsin.
- ⑦ Light hits the rod cell causing the cleavage of rhodopsin.
- ⑧ All-trans retinol is first converted to all-trans retinol before being ultimately converted back to 11-cis retinal.
- ⑨ All-trans retinol attaches to IRBP for transport across the interphotoreceptor space and into the pigment epithelium.
- ⑩ All-trans retinol is released from IRBP and attaches CRBP in the pigment epithelium to enter the cycle again at ②.

Figure 10.7 The visual cycle.

cell differentiation and inhibits the cell cycle. Epithelial cells are one example of cells that rely on vitamin A. Epithelial cells are found as part of our skin and in all internal body tracts, such as the respiratory, gastrointestinal, and urogenital tracts. Retinoic acid helps maintain both the normal structure and the functions of the epithelial cells. For example, retinoic acid directs the differentiation of **keratinocytes** (immature skin cells) into mature epidermal cells. Retinoic acid is thought to act as a signal to “switch on” the genes for keratin proteins [12]. Vitamin A appears to direct the synthesis of keratins, with genes for smaller (versus larger) keratin molecules transcribed and translated in the presence of vitamin A [15]. Vitamin A, *in vitro*, directs differentiation of squamous epithelial keratinizing cells into mucus-secreting cells. With vitamin A deficiency, keratin-producing cells replace mucus-secreting cells in many body tissues.

In addition to epithelial cells, retinoic acid is thought to regulate proliferation and differentiation of myeloid precursors. With adequate vitamin A, myeloid progenitors differentiate into myeloid dendritic cells [17]. Dendritic cells present antigens to other immune system cells, such as T-cells, to augment the body's immune response.

Retinoids also have been shown to induce arrest of the cell cycle and differentiation in cancer and other cell lines. In hematopoietic cells, retinoic acid mediates insulin receptor substrate (IRS) 1 levels by stimulating the binding of the ubiquitin-proteasomal complex to IRS 1; this attachment induces the degradation of IRS 1. IRS 1 regulates cell proliferation and survival in selected cells.

Gene Expression The mechanism by which retinoic acid is thought to affect cell differentiation among other body functions, at least in part, is through its effects on gene

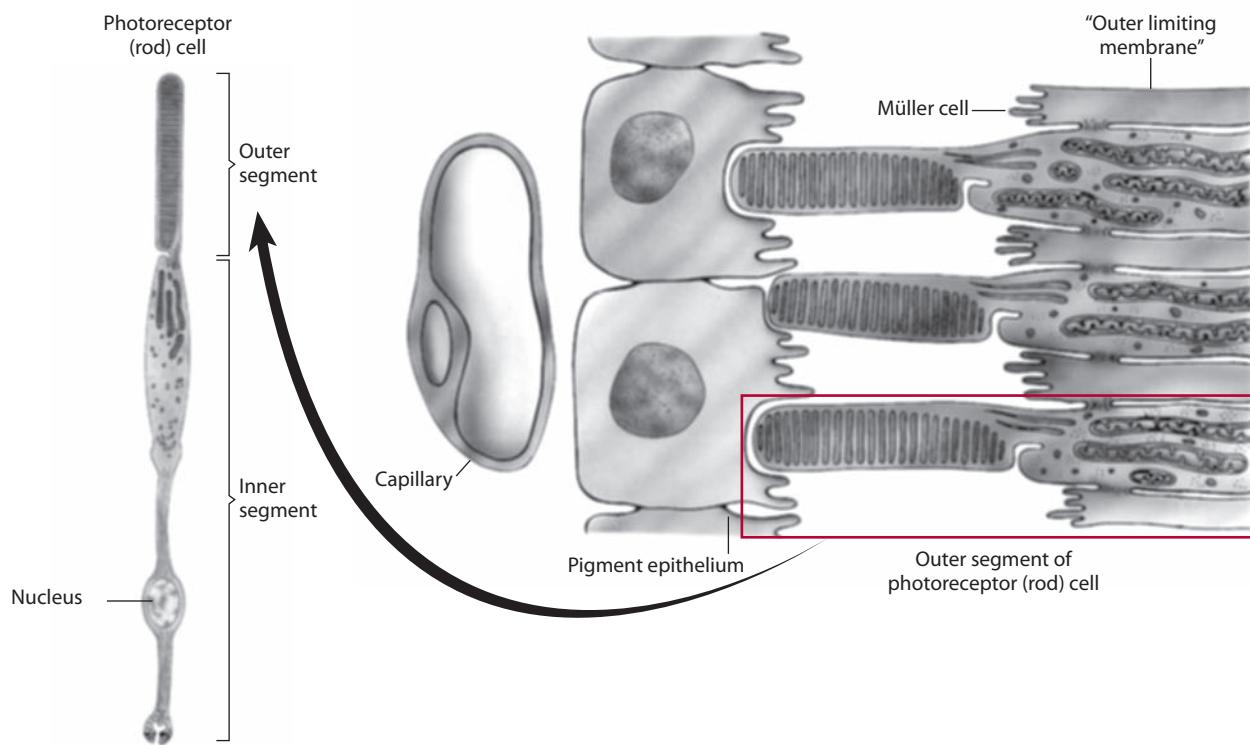


Figure 10.8 Photoreceptor (rod) cells, their structure, and their surroundings.

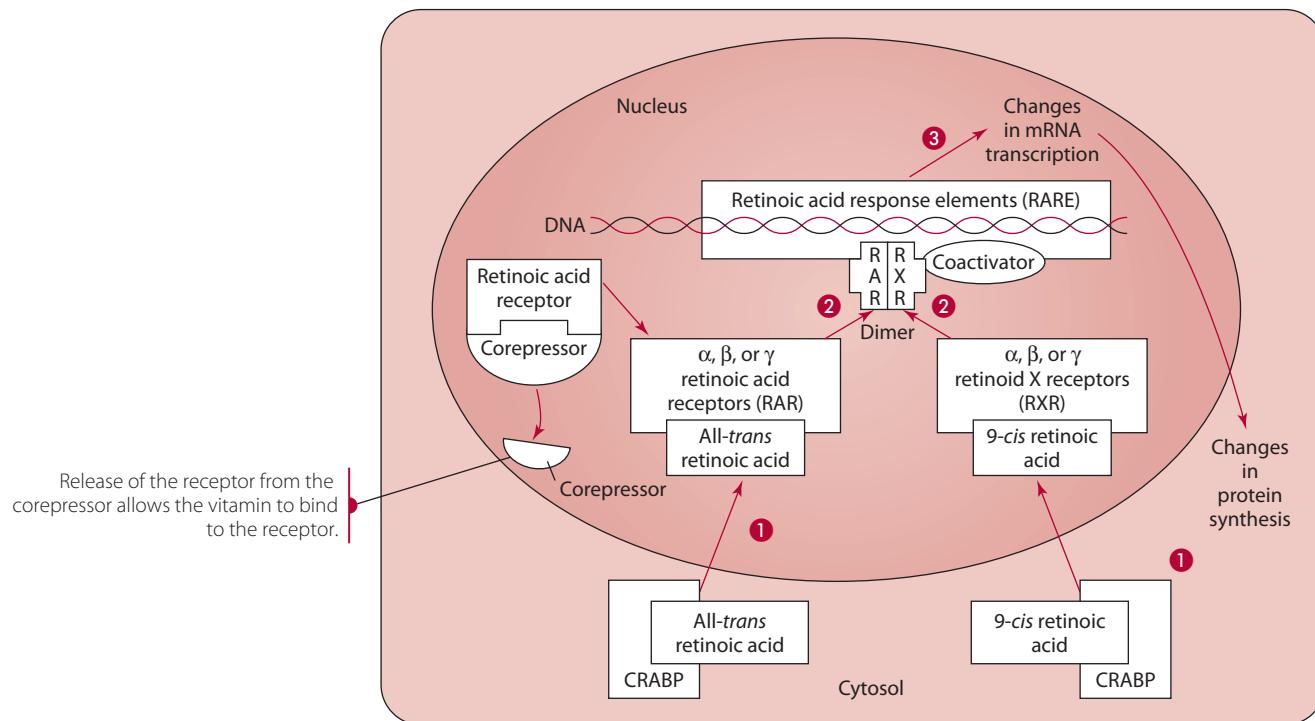
expression (Figure 10.9). A series of events move the retinoic acid into the nucleus, where it interacts with the DNA. First, all-*trans* retinoic acid or 9-*cis* retinoic acid (generated from 9-*cis* retinol) is transported into the nucleus, likely bound to CRABP. Within the nucleus, all-*trans* retinoic acid and 9-*cis* retinoic acid bind to retinoic acid receptors. These nuclear retinoic acid receptors are part of a family of receptors that also includes receptors for vitamin D, specifically calcitriol/1,25-(OH)₂D₃; for thyroid hormones; and for steroid hormones. Within the nucleus, all-*trans* retinoic acid (and possibly 9-*cis* retinoic acid) binds to one or more of three (α , β , γ) retinoic acid receptors (RAR), and 9-*cis* retinoic acid binds to one or more of three (α , β , γ) retinoid X receptors (RXR). RAR may require release from a corepressor before binding by all-*trans* retinoic acid.

Once formed, the vitamin A–receptor complexes typically interact with other receptors, transcription factors, or coactivator proteins (which may include vitamin D receptors, thyroid hormone receptors, peroxisome proliferator-activated receptors, or orphan receptors with no known ligand) to form various **homodimers** or **heterodimers**. A homodimer is formed when two of the same receptors interact, such as RAR-RAR or RXR-RXR. A heterodimer is formed between two or more different receptors, such as RXR-RAR or VDR (vitamin D receptor)-RXR. The resulting homodimer or heterodimer complexes bind to specific DNA nucleotide sequences, called retinoic acid response elements (RARE), in promoter regions of specific genes. The roles and effects on gene expression of these vitamin A-containing complexes are mostly

unknown, but the complexes are thought to affect a wide variety of cellular processes (including cell death, or apoptosis) and body processes through effects on genes that code for enzymes and for growth and transcription factors, among others.

Growth Vitamin A deficiency has long been characterized in animals by impaired growth that can be stimulated with replacement by either retinol or retinoic acid. Specifically, vitamin A stimulates the growth of epithelial cells. Retinyl β -glucuronide, formed in a variety of tissues from retinol and UDP-glucuronic acid (Figure 10.10), actively supports growth and differentiation, although this compound (retinyl β -glucuronide) is also a form of the vitamin used for the vitamin's excretion in the bile [18].

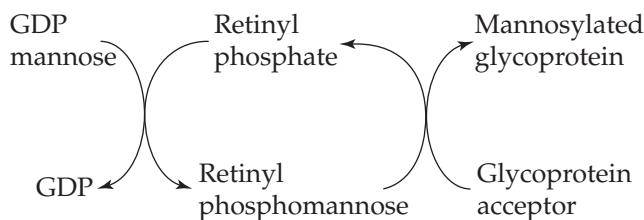
The exact mechanism by which vitamin A affects growth is unclear. Cell growth is stimulated, in part, by growth factors that bind to specific receptors on cell surfaces. Retinoic acid appears to increase the number of specific receptors for growth factors. Retinoic acid and 4-oxoretinoic acid (Figure 10.4) generated from retinoic acid also have been shown to increase synthesis of a specific gap junction protein known as connexin 43 by stabilizing connexin 43 mRNA [19]. **Gap junctions**, cell-to-cell channels formed from connexin proteins, are important for the exchange of small signaling compounds and thus for cell-to-cell communication. A lack of gap junction communication results in uncontrolled cell growth, as can occur with cancer cells. Thus, vitamin A, in preserving this communication, plays a role in the control of cell growth.



- ① All-trans or 9-cis retinoic acid moves into the nucleus of the cell.
- ② All-trans retinoic acid binds to retinoic acid receptors (RAR) and 9-cis retinoic acid binds to retinoid X receptors (RXR). These vitamin-bound receptors attach to specific sites on the DNA referred to as retinoic acid response elements (RARE), found in the promoter region of specific genes.
- ③ Binding of the receptors to RARE on the DNA enhances the transcription of selected genes.

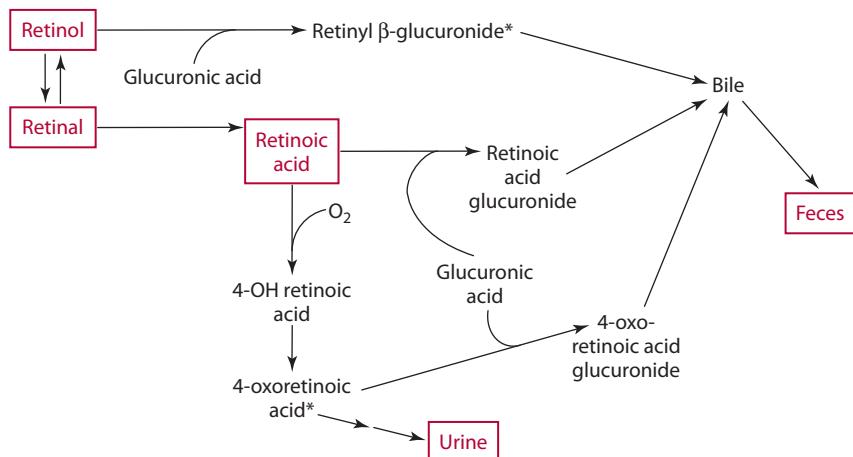
Figure 10.9 Hypothesized mode of action for retinoic acid on gene expression.

Retinoic acid also may be able to modify cell surfaces, possibly by increasing glycoprotein synthesis at the gene level or by improving the attachment of glycoproteins to cell surfaces to induce cell adhesion [18]. Retinol may also play a more direct role in glycoprotein synthesis. Retinyl phosphate (generated from retinol plus ATP) can be converted to retinyl phosphomannose (also called mannosyl retinyl phosphate) in the presence of GDPmannose. Retinyl phosphomannose can in turn transfer the mannose to glycoprotein acceptors. On receipt of the mannose, the glycoprotein acceptors become mannosylated glycoproteins [18]. Such changes in the glycan portion of the glycoprotein can greatly affect differentiation of cells or tissues through their effects on cell recognition, adhesion, and cell aggregation. These reactions involving vitamin A and glycosylation are shown here.



Other Functions Vitamin A, as retinol but not as retinoic acid, is essential for reproductive processes in both males

and females, although the mechanism(s) of its action(s) are unclear [20]. Bone development and maintenance also require vitamin A. Vitamin A is necessary for bone metabolism through involvement with **osteoblasts** (bone-forming cells) and **osteoclasts** (cells involved in bone resorption). Although the mechanism of action is unclear, vitamin A deficiency results in excessive deposition of bone by osteoblasts and reduced bone degradation by osteoclasts. Excess vitamin A, in contrast, stimulates osteoclasts and inhibits osteoblasts, decreasing bone mineral density and increasing fracture risk. Vitamin A appears to be involved in hematopoiesis and iron distribution among tissues, also by an unknown mechanism of action. Several aspects of immune system function, both humoral and cell-mediated, also are influenced by vitamin A. Retinoic acid, for example, stimulates phagocytic activity and cytokine production and maintains natural killer cell concentrations [21]. Depletion studies suggest that vitamin A appears to be needed for T-lymphocyte function and for antibody response to viral, parasitic, and bacterial infections [21]. Thus, people with vitamin A deficiency have an impaired ability to resist and fight infections. Another role of vitamin A, likely mediated by effects on gene expression, and cell differentiation and growth, is in morphogenesis/embryogenesis. Specifically, retinoic acid is thought to act as a morphogen in embryonic developments, and nuclear



*May function in the body in growth, development, and cell-to-cell communication

Figure 10.10 Metabolism of vitamin A, showing some excretory products that are secreted into the bile for removal from the body.

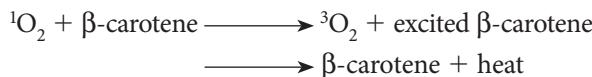
retinoic acid receptors have been found in different cells during different times of development in the embryo.

Carotenoids

Carotenoids are thought to be present in the interior membranes of cells as well as in lipoproteins. Structurally, carotenoids possess an extended system (often nine or more) of conjugated double bonds that make them soluble in lipids and capable of quenching **singlet molecular oxygen** (1O_2) and of **free radicals** (atoms or molecules with one or more unpaired electrons). In other words, carotenoids are thought to function as antioxidants, because they possess the ability to react with and quench free-radical reactions in lipid membranes or compartments and possibly in solution. In addition to antioxidant roles, studies with carotenoids suggest roles in cell proliferation, growth, and differentiation and in enhancing cell-mediated immune functions. Some proposed functions of carotenoids and their relationship to diseases are discussed next.

Antioxidant Functions Quenching is a process by which electronically excited molecules, such as singlet molecular oxygen, are inactivated. Singlet molecular oxygen possesses higher energy and is more reactive than ground state molecular oxygen, which typically exists in triplet (3O_2) rather than singlet (1O_2) form. Singlet oxygen is generated from lipid peroxidation of membranes, transfer of energy from light (photochemical reactions), or the respiratory burst occurring in neutrophils, for example (enzymatic reactions). Singlet molecular oxygen readily reacts with organic molecules such as protein, lipids, and DNA and thus can damage cellular components unless removed. Carotenoids such as β -carotene or lycopene can react with (quench) singlet oxygen, and it is the conjugated double-bond systems within the carotenoids that permit the quenching. For example, β -carotene bound to lymphocytes taken from human blood directly quenches singlet molecular oxygen in vitro [22]. Lycopene appears to have the highest rate constant (i.e., ability to react or

complex) and is a more effective quencher of singlet oxygen than other carotenoids [23]. The singlet oxygen (1O_2) transfers its excitation energy and returns to the ground state (3O_2), and the carotenoid receiving the energy enters an excited state. Resonance states in the excited carotenoid allow some stabilization. Carotenoids then release the energy in the form of heat and thus do not need to be regenerated.



In addition to quenching singlet oxygen, β -carotene and other carotenoids have the ability to react directly with **peroxyl radicals** (O_2^-) involved in lipid peroxidation. This ability has been demonstrated in the range of partial pressures of oxygen that exist under physiological conditions. Studies suggest that β -carotene works synergistically with vitamin E in scavenging radicals and inhibiting lipid peroxidation, although vitamin E has higher reactivity toward peroxyl radicals than does β -carotene [24,25]. β -carotene is thought to function in the interior of the membrane, whereas α -tocopherol functions on or at the surface of the membrane [24]. Lipid peroxidation has been significantly reduced in smokers (who have chronic oxidative damage from smoking) receiving only 20 mg β -carotene for 4 weeks [26]. β -carotene supplementation (50–100 mg daily for 3 weeks) shortens the lag phase of metal (copper)-dependent lipid peroxidation of LDLs. In other words, β -carotene supplementation helps make LDL more resistant to metal-induced lipid oxidation. Lipid peroxidation is an indicator of free radical activity and reflects damage to membranes and possibly other organelles or DNA. Supplements providing 120 mg β -carotene also significantly reduced lipid peroxidation in humans [27]. Carotene depletion in humans results in significantly lower serum carotene concentrations and higher concentrations of **thiobarbituric acid-reactive substances**, such as hexanal, pentanal, and pentane (compounds that indicate oxidative damage) [28]. Repletion studies providing

β -carotene (15 or 120 mg) have been shown to decrease the concentrations of circulating peroxides [28,29].

Although most studies have focused on β -carotene, lycopene (in chemical assays) has been shown to be the strongest antioxidant [30]. In these *in vitro* assays, lycopene is followed in descending order by α -tocopherol (vitamin E), α -carotene, β -cryptoxanthin, zeaxanthin and β -carotene, and lutein [30]. Combinations of the carotenoids, however, especially lycopene and lutein, have been shown to work synergistically and thus are more effective than any one of the carotenoids by itself.

Because of the ability of carotenoids to react with free radicals and quench singlet oxygen, carotenoids are thought to be protective against several diseases. Epidemiological studies have shown that people with high intake of fruits and vegetables, which are also rich in carotenoids, or people with higher serum carotenoid concentrations have a lower incidence of diseases such as cardiovascular disease, cancer, cataracts, and age-related macular degeneration [31–39].

Carotenoids and Eye Health Age-related macular degeneration is a common cause of blindness in older people. The macula, found in the center of the retina, maintains central vision. Lutein and zeaxanthin, which are found in the macula, can inhibit oxidation of cell membranes and thus may be protective against UV-induced eye damage. Studies have demonstrated that people in the highest quintile of intake of dietary carotenoids, especially lutein and zeaxanthin, and those with high serum lutein and zeaxanthin concentrations have significantly lower risk of macular degeneration [36,37]. In addition, in one study lutein supplementation (10 mg) along with other antioxidants appeared to improve visual function in some with age-related macular degeneration. The Food and Drug Administration, however, concluded (in 2006) that no credible evidence exists to allow a health claim for intake of lutein or zeaxanthin as a way to reduce risk of age-related macular degeneration or cataracts [40].

Carotenoids and Heart Disease In the mechanism(s) thought to lead to the development of atherosclerosis, oxidation of cholesterol in LDLs increases the likelihood of monocyte or macrophage uptake in comparison with native (unoxidized) cholesterol. Oxidized cholesterol also impairs arterial nitric oxide release, which may contribute to vasospasms and platelet adhesion associated with heart disease. Oxidized LDL cholesterol stimulates the binding of monocytes to blood vessel endothelium. These monocytes become embedded in the endothelium, as do native LDLs, which have the potential to become oxidized within the subendothelial space. Macrophages in the blood vessel endothelia continue to take up oxidized LDL cholesterol through a specific cell surface scavenger receptor and, when filled with cholesterol, become foam cells. Fatty streaks (an accumulation of foam cells) lead to atherosclerotic

plaques in blood vessels. Carotenoids, including β -carotene, lycopene, lutein, and α -carotene, are thought to prevent the oxidation of LDL cholesterol and other cell membrane lipids and thus prevent or slow the development of atherosclerosis. However, α -tocopherol (vitamin E) may be more effective than β -carotene in inhibiting LDL oxidation [41]. Supplementation with 800 IU vitamin E, 1,000 mg vitamin C, and 24 mg β -carotene in people with coronary artery disease effectively reduces LDL susceptibility to oxidation [42]. Yet, the effectiveness of carotenoids alone in preventing or treating heart disease has not been established, and, at present, carotenoid supplementation is not recommended [43].

Cell Proliferation, Growth, and Differentiation Carotenoids inhibit cell proliferation and stimulate cell differentiation while also affecting growth. Lycopene, for example, inhibits the growth and proliferation of various cancer cells and induces cell differentiation [44,45]. *In vitro*, canthaxanthin and β -carotene have been shown to inhibit carcinogen-induced neoplastic transformation as well as to inhibit lipid oxidation in plasma membranes [46]. As in their antioxidant roles, carotenoids appear to vary in their abilities to inhibit cell transformation. For example, canthaxanthin appears to be more effective than β -carotene, followed by α -carotene and then lycopene, in inhibiting chemically induced neoplastic transformation [47]. The mechanisms by which carotenoids carry out these roles are not clear, but in their capacities they may be similar to the retinoids. For example, carotenoid-induced enhancement or induction of gap junction communication, similar to that of retinoic acid, has been proposed. Some carotenoids up-regulate the gene expression of connexin 43 [46]. Connexin proteins enable connections (bridges) to form between cells, and these connections in turn affect cell proliferation and growth [19]. Gap junction communications are inhibited in some cancer cells. Thus, the ability of carotenoids to protect this activity may be of significance in cancer prevention [48].

Carotenoids and Cancer Intervention trials using β -carotene have failed to show protective effects on health. Intervention trials with 20 mg of β -carotene (BC) along with α -tocopherol (AT) in Finland (called the ATBC trial) as well as intervention with 30 mg of β -carotene and 25,000 IU of vitamin A (CARET trial) showed no benefit over placebo in cancer prevention in asbestos-exposed workers and in people with a long history of smoking [49,50]. β -carotene taken with the vitamin A even appeared to increase the risk of lung cancer and increase the risk of mortality from cancer and heart disease in the high-risk populations (smokers and asbestos-exposed) [50]. These two intervention trials appear to suggest that β -carotene supplementation increases the risk of cancer; however, others suggest that the populations were at high risk for disease, with a long history of smoking (one pack or more

cigarettes daily) or asbestos exposure along with alcohol consumption, and that only with these cofactors does β -carotene supplementation increase cancer risk [51,52].

Carotenoids and Health Claims Health claims that are approved by the U.S. Food and Drug Administration are targeted at carotenoid-rich foods (fruits and vegetables) as well as grain products. These claims require the food to be low in fat and a good source of dietary fiber without fortification. An example model claim is “Low fat diets rich in fiber-containing grain products, fruits and vegetables may reduce the risk of some types of cancer, a disease associated with many factors” [53]. Another sample claim for fruits, vegetables, or grain products that are low in (saturated and total) fat and cholesterol and contain at least 0.6 g soluble fiber per reference amount without fortification is “Diets low in saturated fat and cholesterol and rich in fruits and vegetables and grain products that contain some types of dietary fiber, particularly soluble fiber, may reduce the risk of heart disease, a disease associated with many factors” [53]. Another model claim—“Low fat diets rich in fruits and vegetables (foods that are low in fat and may contain dietary fiber, vitamin A, or vitamin C) may reduce the risk of some types of cancer, a disease associated with many factors”—has also been approved for fruits and vegetables that are a good source of vitamin A or C or dietary fiber [53].

INTERACTIONS WITH OTHER NUTRIENTS

Vitamin A or carotenoids interact with vitamins E and K. Excess dietary vitamin A intake interferes with vitamin K absorption. High β -carotene intake, in turn, may decrease plasma vitamin E concentrations.

Protein and zinc influence vitamin A status and transport. First, the activity of the enzyme carotenoid dioxygenase that cleaves β -carotene is depressed by inadequate protein intake. Second, overall vitamin A metabolism is closely related to protein and zinc status because transport and use of the vitamin depend on several vitamin A-binding proteins and because zinc is required for protein synthesis. Impairments in the synthesis of proteins, including retinol-binding protein and transthyretin, diminish retinol mobilization from the liver. In addition, in peripheral tissue, alcohol dehydrogenase, which converts retinol into retinal, is directly dependent on zinc.

Iron status and vitamin A are also interrelated. The effects may be mediated through a role played by vitamin A in hematopoiesis. Vitamin A deficiency is associated with decreased iron incorporation into red blood cells and diminished mobilization of iron from stores (and thus accumulation of iron in some tissues). Thus, vitamin A deficiency can be associated with microcytic iron deficiency anemia. Vitamin A supplementation, in turn, corrects the anemia, with observed improvements in indices of iron status [54–57]. Vitamin A (likely as retinoic acid)

may be directly acting on iron metabolism or storage or may be affecting differentiation of blood cells [58].

METABOLISM AND EXCRETION

Retinol, as well as retinoic acid, is typically oxidized at the β -ionone ring and then conjugated to generate polar, water-soluble metabolites. The kidney is the main organ responsible for the catabolism and excretion of the vitamin. Urinary excretion of vitamin A metabolites accounts for up to about 60% of vitamin A excretion. In addition, small amounts of vitamin A are expired by the lungs as CO_2 , and up to about 40% of vitamin A metabolites are excreted in the feces [3]. Fecal vitamin A metabolites are typically those oxidized products of vitamin A that contain intact chains and have been conjugated to glucuronic acid (Figure 10.10) or taurine and excreted into the bile. Some examples of fecal vitamin A metabolites are retinoic acid glucuronide and 4-oxoretinoic acid glucuronide. However, some polar products such as 4-oxoretinoic acid glucuronide can be absorbed and returned to the liver through the enterohepatic circulation. This recycling mechanism helps conserve the body’s supply of vitamin A when necessary.

Carotenoids newly absorbed and not stored or converted to retinal or retinol may be metabolized into a variety of compounds depending on the individual carotenoid. Carotenoid metabolites are excreted into the bile for elimination in the feces [18].

RECOMMENDED DIETARY ALLOWANCE

In 2001, the Institute of Medicine’s Food and Nutrition Board published new recommendations for vitamin A; however, no recommendations were established for carotenoids because the carotenoids are not considered essential nutrients [3]. Recommendations for vitamin A intake are expressed as retinol activity equivalents (RAE). Older units of measure include international units (IU) and retinol equivalents (RE). The RAE equivalencies of retinol, β -carotene, and other pro-vitamin A carotenoids are as follows [3]: 1 RAE = 1 μg retinol = 12 μg β -carotene = 24 μg α -carotene or β -cryptoxanthin.

The requirements for vitamin A for adult men and women are 625 and 500 μg RAE, respectively [3]. The recommended dietary allowance (RDA) for vitamin A is based on the requirement plus twice the coefficient of variation (20%) with the value rounded to the nearest 100 μg [3]. The RDAs for vitamin A for adult men and women are 900 and 700 μg RAE, respectively [3]. During pregnancy and lactation, the RDAs for vitamin A for adult women are higher at 770 and 1,300 μg RAE, respectively [3]. A daily tolerable upper intake level (UL) for adults of 3,000 μg has been established for preformed vitamin A [3].

Because international units are still found on food labels, conversion factors are listed hereafter. One IU vitamin A

is equal to 0.3 µg retinal, 3.6 µg β carotene, and 7.2 µg of other provitamin A carotenoids.

DEFICIENCY

Vitamin A deficiency is less common in the United States than in developing countries, where inadequate intake is fairly common in children under 5 years of age. In developing countries, increased mortality is associated both with clinically evident vitamin A deficiency in children and with inadequate vitamin A stores in children with no clinical signs of deficiency [59]. Increased infectious morbidity also is associated with vitamin A deficiency [21].

Selected signs and symptoms of deficiency include xerophthalmia, anorexia, retarded growth, increased susceptibility to infections, obstruction and enlargement of hair follicles, and keratinization of epithelial (mucous) cells of the skin with accompanying failure of normal differentiation. **Xerophthalmia** is characterized by dryness of the eye (because of inadequate mucus production) and abnormalities of the conjunctiva and cornea of the eye; it includes conjunctival and corneal xerosis, Bitot's spots, corneal scarring and ulcerations, and night blindness. Conjunctival changes include the disappearance of goblet cells in the conjunctiva, the enlargement and keratinization of epithelial cells, and the appearance of Bitot's spots overlying the keratinized epithelia of the conjunctiva. **Bitot's spots** are white accumulations of sloughed cells and secretions. Keratomalacia may occur if the changes in the cornea become severe and irreversible (such as with corneal perforation and loss of aqueous humor). Night blindness, which may be one of the first symptoms of vitamin A deficiency related to vision, results from impaired production of rhodopsin in the rod cells in the retina of the eye.

Conditions and populations associated with increased need for vitamin A include people with malabsorptive disorders such as **steatorrhea** (excessive fat in the feces) or pancreatic, liver, or gallbladder diseases. People with chronic nephritis, acute protein deficiency, intestinal parasites, or acute infections may also become vitamin A-deficient. Measles infections in developing countries are associated with high mortality. Measles is thought to depress vitamin A status (which may already be low in children in developing countries) by diminishing vitamin A intake, absorption, and use and by increasing urinary vitamin A excretion [21]. Vitamin A supplements are recommended by WHO and UNICEF for children with measles living in a country with measles fatality rates of 1% or greater [19].

TOXICITY: HYPERVITAMINOSIS A

Ingesting a large amount of vitamin A in a short time (even a single dose) may result in acute hypervitaminosis A. Symptoms of acute hypervitaminosis A include nausea, vomiting, double vision, headache, dizziness, and general

desquamation of the skin. Chronic intake (daily for months or years) of lower doses in excess of recommended amounts also can lead to hypervitaminosis A. In adults, chronic oral intake of vitamin A (retinol) in amounts as little as 3 to 4 times greater than the RDA can result in hypervitaminosis A, although generally a higher intake (about 10 or more times the RDA) is required to result in toxicity. Chronic vitamin A toxicity is manifested by a variety of maladies, including anorexia; dry, itchy, and desquamating skin; alopecia (hair loss) and coarsening of the hair; ataxia; headache; bone and muscle pain; increased bone fractures; conjunctivitis and ocular pain, and liver damage. Most manifestations of toxicity appear to subside gradually once excessive intake of the vitamin is discontinued [60]. See Penniston and Tanumihardjo [61] for a review of the acute and chronic toxic effects of vitamin A. The tolerable upper intake level for preformed vitamin A is 3,000 µg (10,000 IU) per day [3], although some studies have suggested that single and repeated oral doses of 30,000 IU vitamin A as vitamin A palmitate by nonpregnant women were without safety concerns [62]. In contrast, others report evidence for adverse effects on bone (increased fracture and decreased bone density) with daily intake greater than 1500 µg RE [63].

Excess vitamin A also is **teratogenic** (causes birth defects). For example, an oral total intake of more than 4,500 µg RE by pregnant women has been associated with increased risk of malformations in infants born to the women [63,64]. Moreover, the oral medication Accutane (an acne treatment), which contains a modified form of vitamin A (13-cis retinoic acid), also is teratogenic. Use of this compound by women in the early months of their pregnancy resulted in a number of birth defects among the infants born to these women. Consequently, dermatologists advise against Accutane usage for women who are or may become pregnant and thus prescribe contraceptives for patients in their childbearing years taking the drug.

One mechanism by which excess vitamin A is thought to exert toxic effects is related to its transport in the blood. When vitamin A intake is in excess, serum retinol levels rise especially above 200 µg/dL (normal is 30–86 µg/dL), and retinol is no longer transported exclusively by RBP but instead can be carried to the tissues by plasma lipoproteins. It has been suggested that when retinol is presented to the cell membranes in a form other than in an RBP complex, the released retinol produces toxic effects [3,16]. Effects on the liver, the primary storage site for vitamin A, are multiple. They include fat-storing cell **hyperplasia** (excessive cell proliferation) and hypertrophy, fibrogenesis, sclerosis of veins, portal hypertension, and congestion in perisinusoid cells, which leads to hepatocellular damage and cirrhosis or a cirrhosis-like hepatic disorder [60,65]. Other toxic effects of excess vitamin A intake are thought to be mediated by changes in the regulation of vitamin A (retinoid) receptors in the nucleus and by effects on cell differentiation and growth, among other means.

Carotenoids, in contrast to vitamin A, appear to have few side effects. In fact, β -carotene is listed on the generally recognized as safe (GRAS) list with the FDA for use as a dietary and nutrient supplement as well as for use as a colorant in foods, drugs, and cosmetics [66]. Ingestion by adults in amounts as high as 180 mg β -carotene daily for several months has been shown to pose no serious side effects [67]. Hypercarotenosis in people ingesting about 30 mg or more of β -carotene daily can, however, cause a yellow discoloration of the skin, especially in the fat pads or fatty areas of the palms of the hands and soles of the feet. The condition usually disappears once the carotenoids are removed from the diet. No tolerable upper intake level has been established for β -carotene or other carotenoids [5], but excessive consumption of carotenoids may be detrimental to smokers [50–52] and possibly to nonsmokers, but effects are unknown at this time. Carotenoid supplements are not advised for the general public; instead, the public is encouraged to consume at least five servings of fruits and vegetables per day [5].

ASSESSMENT OF NUTRITION

Vitamin A status may be assessed in a variety of ways, including histological tests, biochemical measures, and functional tests. These tests include assessing for night blindness or dark adaptation threshold. Electrophysiological measurements made by electroretinograms measure the level of rhodopsin and its rate of regeneration.

Conjunctival impression cytology (CIC), a histological method of assessment, involves examining the eye. The conjunctiva is examined for changes, specifically a reduction in goblet cells and the derangement of epithelial cells, that occur with impaired vitamin A status [68].

Plasma retinol concentrations are frequently measured as a biochemical indicator of vitamin A status. Plasma retinol levels reflect status best if the person has exhausted his or her stores (primarily in the liver) of the vitamin, as with deficiency, or if the stores are filled to capacity, as with toxicity. Use of plasma retinol concentrations, however, also depends on the adequacy of dietary energy, protein, and zinc because of their roles in the synthesis of retinol-binding protein. Moreover, use of plasma retinol is unreliable as an indicator of vitamin A status in people with infection or inflammation, both of which depress plasma concentrations of the vitamin [67]. Plasma retinol concentrations less than ~20 $\mu\text{g}/\text{dL}$ (0.7 $\mu\text{M}/\text{L}$) are usually considered as deficient or marginal and suggestive of inadequate stores of vitamin; although others suggest plasma retinol concentrations <10 $\mu\text{g}/\text{dL}$ (0.35 $\mu\text{M}/\text{L}$) indicates deficiency, and 10–20 $\mu\text{g}/\text{dL}$ (0.35–0.7 $\mu\text{M}/\text{L}$) suggests marginal status [3,69,70]. Plasma retinol concentrations of 30–86 $\mu\text{g}/\text{dL}$ (1.05–3 $\mu\text{M}/\text{L}$) are thought to be adequate, and concentrations in excess of 86 $\mu\text{g}/\text{dL}$ (3 $\mu\text{M}/\text{L}$) are thought to be excessive.

Adequacy of vitamin A stores in the liver can be assessed by other biochemical tests: the relative dose response (RDR) test or the modified relative dose response (MRDR) test. The RDR test involves measuring changes in plasma retinol concentration before and 5 hours after oral administration of retinyl esters (usually as acetate or palmitate). Blood is taken initially, then vitamin A is ingested, and 5 hours later blood is taken again. Retinol concentrations of the blood are determined, and the difference in concentration is calculated and divided by the 5-hour concentration. RDR is then expressed as a percentage.

$$\text{RDR (\%)} =$$

$$\frac{\text{5-hour plasma retinol} - \text{initial plasma retinol}}{\text{5-hour plasma retinol concentration}} \times 100$$

A % RDR equal to or greater than 20% suggests inadequate liver vitamin A stores [3,71,72]. The MRDR test involves measuring the ratio of 3,4 didehydroretinol to retinol in the blood after administering a single dose of 3,4 didehydroretinyl acetate. This test, unlike the RDR, requires that only one blood sample be taken, about 4 to 6 hours after the vitamin is ingested. An MRDR ratio at 5 hours of less than 0.04 in healthy adults indicates adequate status [73].

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Vitamin D

Through the years vitamin D has been associated with skeletal growth and strong bones. This association arose because early in the 20th century it was shown that rickets, a childhood disease characterized by improper bone development, could be prevented by a fat-soluble factor D in the diet or by body exposure to ultraviolet light. Emphasis was placed on the dietary factor; therefore, any compound with curative action on rickets was designated as vitamin D. E. McCollum is credited with the discovery of vitamin D.

Structurally, vitamin D is derived from a steroid and is considered to be a seco-steroid because one of its four rings is broken. Vitamin D contains three intact rings (A, C, and D) with a break between carbon 9 and 10 in the B ring (Figure 10.11). The vitamin and its metabolites exhibit an unusual conformational flexibility that allows them to effectively interact with binding proteins [1,2].

SOURCES

Dietary vitamin D is provided primarily by foods of animal origin, especially liver, beef, veal, and eggs (mainly the yolk); dairy products such as milk, cheese, and butter; and some saltwater fish, including herring, salmon, tuna, and sardines. In the United States, selected foods, such as milk, yogurt, cheese, and margarine as well as some orange juice, breads, and cereals, are fortified with vitamin D. Milk and orange juice, for example, are fortified with 2.5 μ g (100 IU) vitamin D/cup in the United States. Table 10.2 provides information on major food sources of the vitamin. Dietary vitamin D is a stable compound not prone to cooking, storage, or processing losses.

In plants, a commonly occurring steroid, ergosterol, can be activated by irradiation to form ergocalciferol (also called vitamin D₂ or ercalciole; Figure 10.11). This is the form of the vitamin that is commonly sold commercially. No ergosterol occurs in animals, but another steroid, 5,7-cholestadienol, commonly called 7-dehydrocholesterol, is found in animals and humans.

The steroid 7-dehydrocholesterol is synthesized in the sebaceous glands of the skin and secreted onto the skin's surface, and it may be reabsorbed into the various layers of the skin. It appears to be uniformly distributed throughout the epidermis and dermis. The conjugated set of double bonds (five to seven) in ring B of 7-dehydrocholesterol allows the absorption of specific wavelengths of light found in the ultraviolet range. Thus, during exposure to sunlight, ultraviolet B (UVB) photons (wavelength ~285 to 320 nm) penetrate into the epidermis and dermis. Some 7-dehydrocholesterol in skin cell plasma membranes absorbs the photons; this event causes ring B to open, forming precholecalciferol (also called previtamin D₃; Figure 10.11). The unstable double bonds in precholecalciferol are rearranged (also called thermal isomerization) after a 2- to 3-day period, resulting in the synthesis of vitamin D₃, also called cholecalciferol or calcidiol (Figure 10.11). Lumisterol is also produced from 7-dehydrocholesterol in the presence of ultraviolet light, whereas tachysterol is generated by further irradiation of previtamin D₃.

Cholecalciferol diffuses from the skin into the blood. It is transported in the blood by an α -2 globulin vitamin D-binding protein (DBP), also called transcalficerin, that is synthesized in the liver. Because neither lumisterol, tachysterol, nor previtamin D₃ has much affinity for the DBP, these compounds typically are lost as the skin sloughs off.

ABSORPTION, TRANSPORT, AND STORAGE

Vitamin D₃ (cholecalciferol) from the diet is absorbed from a micelle, in association with fat and with the aid of bile salts, by passive diffusion into the intestinal cell. About 50% of dietary vitamin D₃ is absorbed. Although the rate of absorption is most rapid in the duodenum, the largest amount of vitamin D is absorbed in the distal small intestine.

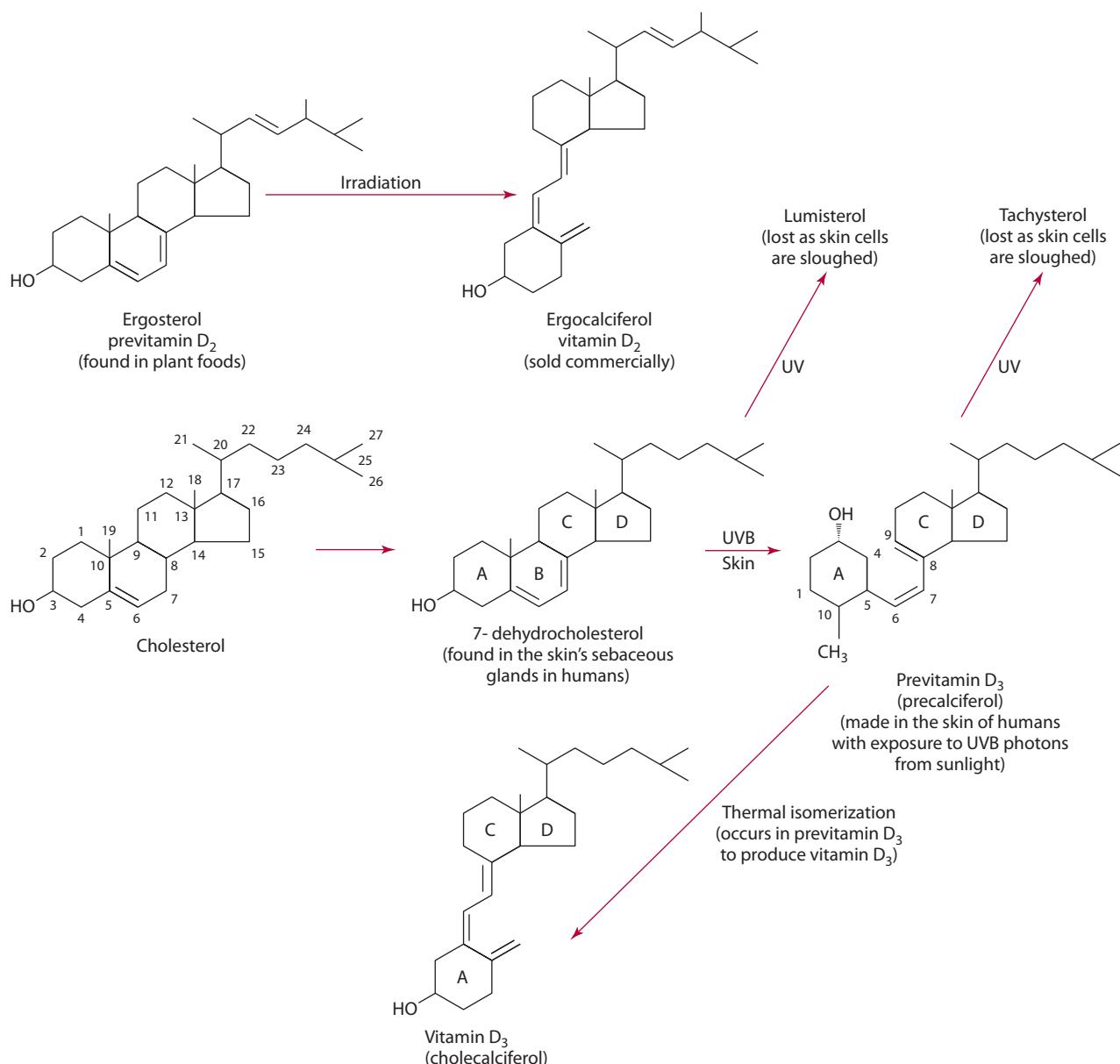


Figure 10.11 Production of ergocalciferol (vitamin D₂) and vitamin D₃ (cholecalciferol).

Table 10.2 Major Food Sources of Vitamin D

Food	Approximate Vitamin D Content ($\mu\text{g}/100 \text{ g}$)
<i>Fortified</i>	
Milk	0.8–1.3
Margarine	8.0–10.0
<i>Nonfortified</i>	
Butter	0.3–2.0
Milk	<1.0
Cheese	<1.0
Liver	0.5–4.0
Fish*	5.0–40.0

*Fatty fish such as herring, salmon, sardines, and tuna.

Within the intestinal cell, vitamin D is incorporated primarily into chylomicrons, which then enter the lymphatic system with subsequent entry into the blood. Chylomicrons transport about 40% of the cholecalciferol in the blood, although some vitamin D may be transferred from the chylomicron to DBP for delivery to extrahepatic tissues. Chylomicron remnants deliver the vitamin to the liver.

Cholecalciferol, which slowly diffuses from the skin into the blood, is picked up for transport by DBP. About 60% of plasma cholecalciferol is bound to DBP for transport. The vitamin D bound to DBP travels primarily to the liver but may be picked up by other tissues, especially muscle and adipose tissue, before hepatic uptake. Thus, the difference in the transport mechanism for cholecalciferol formed in

the skin and that absorbed from the digestive tract affects the distribution of the vitamin in the body.

Cholecalciferol reaching the liver either by way of chylomicron remnants or by DBP typically is metabolized by a couple of different hydroxylases to generate the active form of the vitamin. Detailed reviews of these cytochrome P₄₅₀ hydroxylases, which are collectively referred to as mixed-function oxidases (the enzymes reduce one atom of molecular oxygen to water and one to the hydroxyl group), are available [3]. In the liver, 25-hydroxylase functions in the mitochondria to hydroxylate cholecalciferol at carbon 25 to form 25-OH (vitamin) D₃, also called calcidiol or 25-OH cholecalciferol (Figure 10.12). The efficiency of the liver 25-hydroxylase in converting cholecalciferol to 25-OH D₃ appears to be related to the concentrations of vitamin D and its metabolites. The NADPH-dependent 25-hydroxylase enzyme is more efficient during periods of vitamin D deprivation than when normal amounts of the cholecalciferol are available. Although the liver expresses

most of the 25-hydroxylase, the enzyme is found in other organs, including the lungs, intestine, and kidneys. Once generated, 25-OH D₃ is most often released into the blood, where it represents the main form of vitamin D.

Circulating concentrations of 25-OH D₃ are considered to reflect vitamin D status, and they vary depending on both dietary vitamin D intake and exposure to sunlight. Lower 25-OH D₃ concentrations, for example, are reported among many healthy people in the winter months because of diminished exposure to the sun and are inversely associated with parathyroid hormone (PTH) concentrations [4,5]. Serum 25-OH D₃ concentrations, which should be maintained above 80 nmol/L (>30 ng/mL), have been shown to significantly correlate with vitamin D intake [5–8].

Most of the 25-OH D₃ synthesized in the liver is secreted into the blood and transported by DBP. Because little 25-OH D₃ remains in the liver and very little of this metabolite is taken up by the extrahepatic tissues, the blood is the largest single pool (storage site) of 25-OH D₃, which

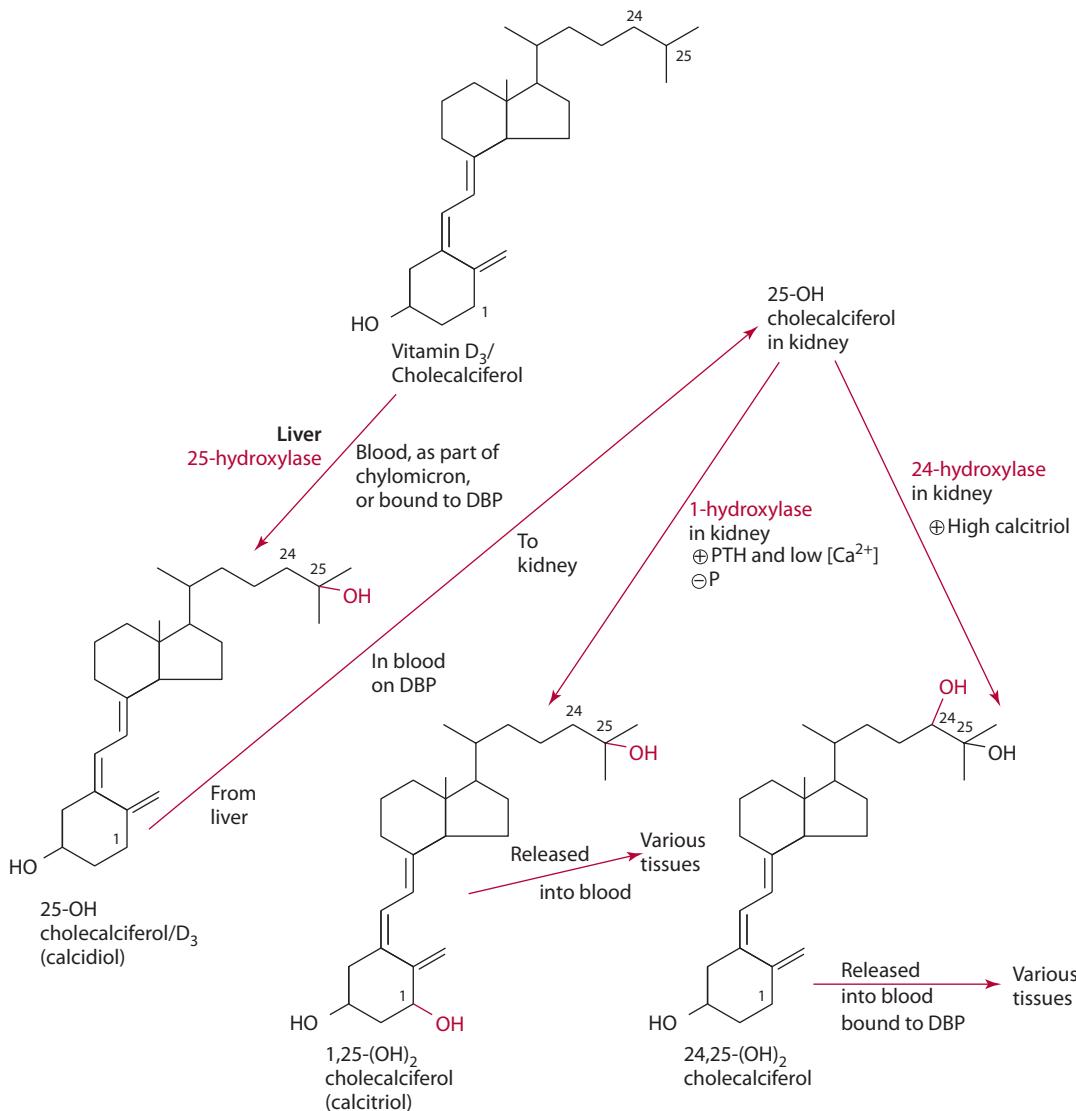


Figure 10.12 Hydroxylation of vitamin D.

has a half-life of about 10 days to 3 weeks [9]. When the 25-OH D₃ pool has been depleted during vitamin D deprivation, maintenance of vitamin D activity is made possible for variable time periods through the release of cholecalciferol from its skin reservoir and from other storage sites. Storage sites for the vitamin are considered to include the blood and muscle (for 25-OH D₃) and adipose tissue (for cholecalciferol).

Following hydroxylation in the liver, 25-OH D₃ bound to DBP is released into the blood and taken up by tissues, especially the kidney. Specifically, the DBP-25-OH D₃ complex binds to megalin on the plasma membrane of the kidney and is transported into renal cells. In the kidney, a second hydroxylation of 25-OH D₃ occurs at position 1, resulting in 1,25-(OH)₂ D₃ (also called 1,25 dihydroxy cholecalciferol, or calcitriol; Figure 10.12), which is considered the active vitamin. Calcitriol is formed in the kidney tubules through the action of an NADPH-dependent mitochondrial enzyme, 1-hydroxylase. This enzyme is expressed in highest concentrations in the kidney but is also present in macrophages, skin, intestine, and bone, among other tissues.

The activity of 1-hydroxylase is influenced by a variety of factors. Parathyroid hormone (PTH) and low plasma calcium concentrations stimulate 1-hydroxylase activity. The concentration of the enzyme's end product, 1,25-(OH)₂ D₃ (calcitriol), also influences the enzyme's activity; high concentrations inhibit 1-hydroxylase activity, and low concentrations stimulate it. Dietary phosphorus intake affects calcitriol production by 1-hydroxylase. A high intake of phosphorus causes a decrease in serum 1,25-(OH)₂ D₃, whereas a low phosphorus intake stimulates its production [10]. When sufficient amounts of calcitriol are present, the activity of 1-hydroxylase in the kidney is decreased significantly, and the activity of another mixed-function oxidase, 24-hydroxylase, is increased in the kidney and possibly in other tissues such as cartilage and the intestine. The vitamin D metabolites 24R,25-(OH)₂ D₃ and 1,24,25-(OH)₃ D₃ are formed from hydroxylation of 25-OH D₃ and 1,25-(OH)₂ D₃, respectively, by 24-hydroxylase (Figure 10.12). Production of 24R,25-(OH)₂ D₃ appears to increase during periods of adequate vitamin D status and calcium homeostasis. The 24R,25-(OH)₂ D₃ form of the vitamin is released into the blood bound to vitamin D-binding protein for functioning at various tissues.

Once synthesized, calcitriol is released from the kidney and bound to DBP for transport in the blood. DBP is one of the major proteins in the blood; the protein transports 1,25-(OH)₂ D₃ along with other metabolites to various target tissues. DBP, however, loosely binds 1,25-(OH)₂ D₃ to facilitate release to tissues, in contrast to its tight binding of 25-OH D₃. Calcitriol in the blood has a half-life of about 4 to 6 hours. On reaching its target tissues, calcitriol is easily released from the DBP and quickly bound by a vitamin D receptor (VDR).

The target tissues of the active vitamin calcitriol initially were believed to be limited to intestine, bone, and kidney, but it is now known that cell membrane receptors for the vitamin are found in many other tissues including cardiac, muscle, pancreas (β -cells), brain, skin, hematopoietic, and immune system tissues [9]. These tissues thus locally produce and use 25-OH D₃ to make 1,25-(OH)₂ D₃ (in other words, 1,25-[OH]₂ D₃ that is made in the tissues is not released into the blood but is used only within the tissues that it is made in). The tissues, however, must have enough 25-OH D₃ (and thus plasma concentrations of 25-OH D₃ must be adequate) to be able to produce enough of the calcitriol.

FUNCTIONS AND MECHANISMS OF ACTION

Calcitriol (1,25-[OH]₂ D₃), the main active form of vitamin D, has several functions and multiple mechanisms of action. Two main mechanisms (genomic and nongenomic) by which calcitriol exerts its functions are known, although the details of the mechanisms have not been clearly elucidated. In some cases, vitamin D is thought to function like a steroid hormone, working through activation of signal transduction pathways linked to cell membrane VDR. In other cases, calcitriol is known to promote genomic actions by interacting with nuclear VDR to influence gene transcription. This section describes both mechanisms of action.

The binding of calcitriol to cell membrane VDR in selected tissues (especially intestine, bone, parathyroid, liver, and pancreatic β -cells) triggers a series of events through intracellular signaling (also called signal transduction) pathways to evoke relatively rapid changes in some body processes. The many actions initiated with this binding include increased intestinal calcium absorption or transcellular calcium flux called **transcalciotaxis** (*trans* means "across," *cal* refers to calcium, and *taxis* means "rapidly") and the opening of gated calcium channels with a resulting increase in calcium uptake into osteoblasts and skeletal muscle cells. These cellular events are thought to be mediated by phosphorylation/dephosphorylation of a number of enzymes and by second messengers such as MAP kinase, protein kinase C, cAMP, tyrosine kinase, phospholipase C, diacylglycerol, inositol phosphate, and arachidonic acid. A membrane-associated rapid response steroid binding (MARRS) protein may also play a role, interacting with a G-protein or other mediator of signal transduction. Additional details on possible vitamin D-mediated signal transduction pathways can be found in the article by Fleet [11].

Nuclear receptors for the vitamin have been found in over 30 organs, including bone, intestine, kidney, lung, muscle, and skin. These vitamin D nuclear receptors are part of a so-called superfamily of receptors that also includes receptors for retinoic acid and thyroid and steroid hormones.

In target tissues, calcitriol binds to nuclear VDR, and this interaction in turn initiates a conformational change in the newly formed complex. The $1,25\text{-}(\text{OH})_2 \text{D}_3$ -VDR complex is phosphorylated and then thought to bind with retinoid X or retinoic acid receptors (RXR or RAR) to form a heterodimeric complex as shown in Figure 10.13. The VDR portion of the heterodimeric complex contains regions with zinc fingers (see Chapter 12, page 494) that can interact with specific vitamin D response elements (abbreviated VDRE), found in the promoter regions of specific target genes (Figure 10.13). Once the heterodimeric complex is bound to the VDRE, additional comodulatory (either coactivator or corepressor) proteins may further interact with the heterodimeric complex to influence (enhance or inhibit) the transcription of genes coding for proteins. The mechanisms by which these comodulatory proteins function are mostly unknown, but they may help link the receptor to enzymes, such as RNA polymerase II, or to other components such as transcription factors necessary for transcription [12]. The comodulatory proteins are thought to include SRC-1, SRC-2, and SRC-3 (the SRC family) as well as NCoA-62 [12]. The proteins ultimately resulting from vitamin D's actions on the genes are typically involved in calcium homeostasis and include, for example, osteocalcin, 24 hydroxylase (CYP24), the epithelial calcium channel transient receptor potential vanilloid-type family member 6 (TRPV6), and calbindin.

As a hormone, one of calcitriol's main functions in the body is acting with parathyroid hormone (PTH) in the homeostasis of blood calcium concentrations. In performing this function, calcitriol and PTH affect several tissues, including intestine, bone, and kidney (Figure 10.14). The effects of calcitriol and PTH on these tissues are discussed next, along with calcitriol's role in cell differentiation, proliferation, and growth.

Calcium Homeostasis

Calcitriol synthesis is stimulated in response to changes in blood calcium concentrations and the release of the

hormone PTH. **Hypocalcemia** (low blood calcium) initially stimulates secretion of PTH from the parathyroid gland. The PTH, in turn, stimulates 1-hydroxylase in the kidney such that 25-OH D_3 is converted to calcitriol. Calcitriol then acts alone or with PTH on its target tissues, causing serum calcium concentrations to rise. The three main target tissues are the intestine, kidney, and bone, as discussed below and shown in Figure 10.14.

Calcitriol and the Intestine A more thoroughly investigated target tissue of calcitriol is the intestine (Figures 10.14 and 10.15). The primary function of calcitriol in the intestine is increased absorption of calcium and phosphorus. The vitamin is believed to interact with both cell membrane receptors and nuclear receptors to affect gene expression and ultimately enhance calcium absorption.

With respect to calcium absorption, calcitriol, as a hormone, interacts with high-affinity vitamin D receptors in the enterocyte and is carried to the nucleus, where it interacts with specific genes encoding for proteins involved in calcium transport. As the result of this interaction, selective DNA transcriptions occur that result in biosynthesis of new messenger RNA (mRNA) molecules. These mRNA molecules are then translated on the endoplasmic reticulum into selected proteins. The proteins may act at the brush border, in the cytoplasm, or at the basolateral membrane of the intestinal cells, especially in the duodenum and jejunum, to promote calcium absorption. For example, calbindin D9k, a calcium-binding protein in the intestinal mucosa, is synthesized in response to the action of calcitriol. Calcitriol also induces the expression of epithelial cell calcium channels. TRPV6 is a calcium channel found in the brush border membrane of the duodenum. Messenger RNA concentrations of TRPV6 appear to be regulated by calcitriol. The process by which vitamin D rapidly initiates intestinal calcium absorption is called transcalcification. The transcalcification response is thought to involve endocytosis of calcium across the brush border membrane, followed by lysosome-mediated release of calcium within the

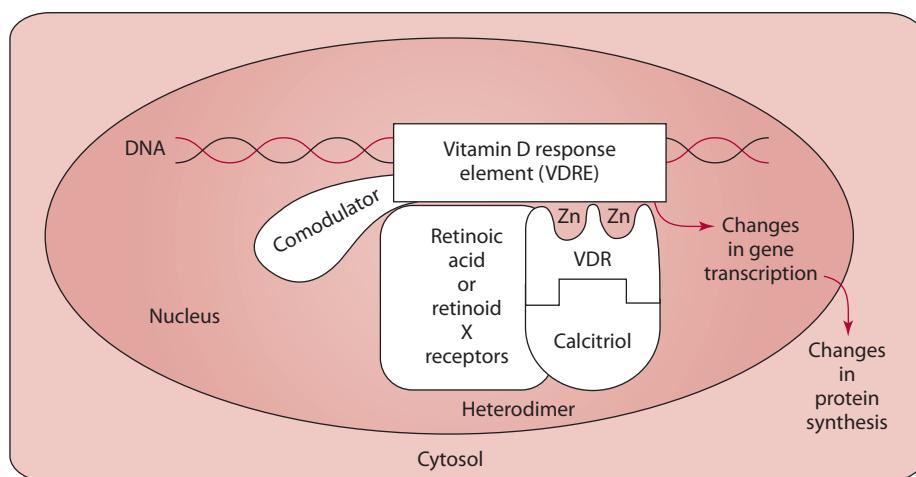


Figure 10.13
Proposed role of calcitriol in gene expression.

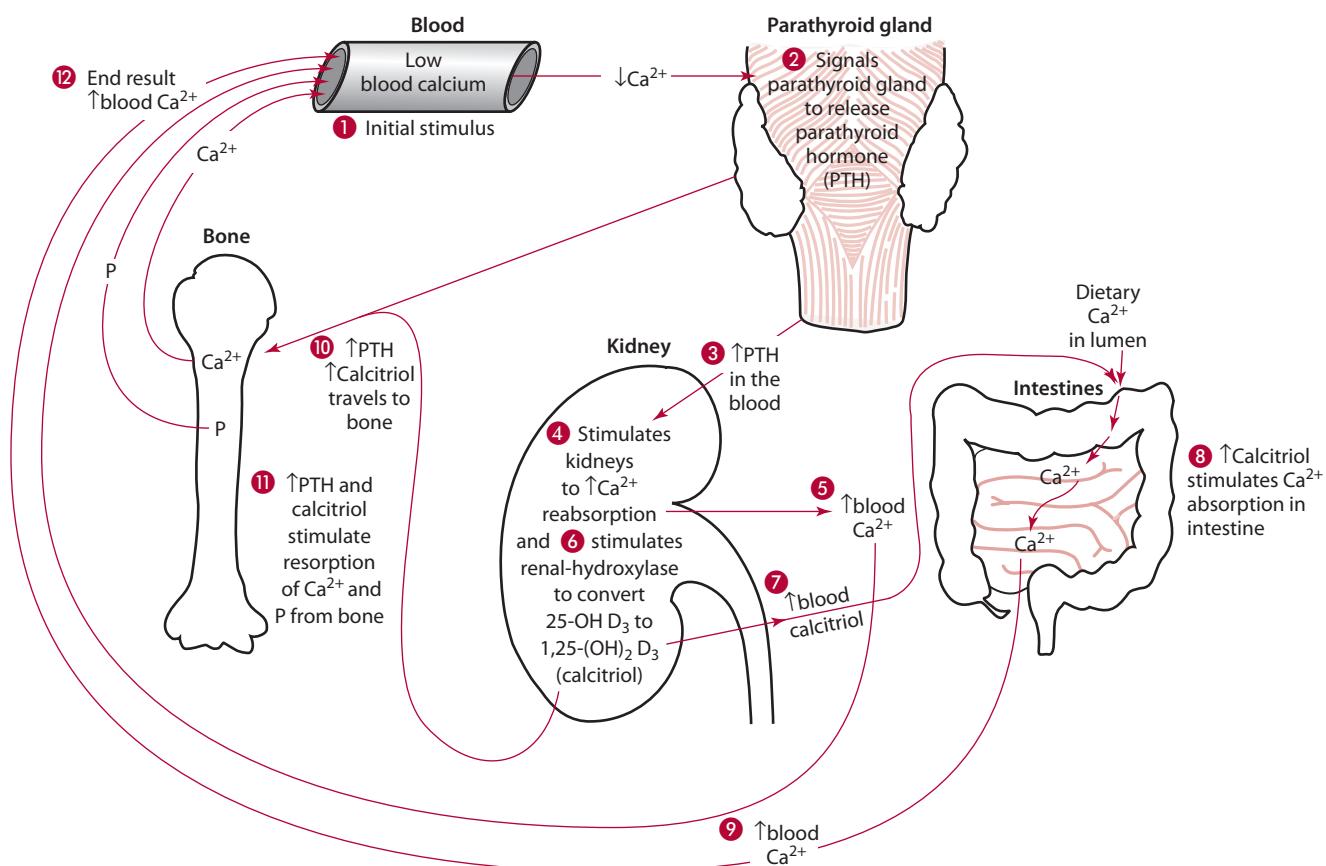


Figure 10.14 Calcitriol, $1,25(\text{OH})_2\text{D}_3$, synthesis and actions with parathyroid hormone (PTH).

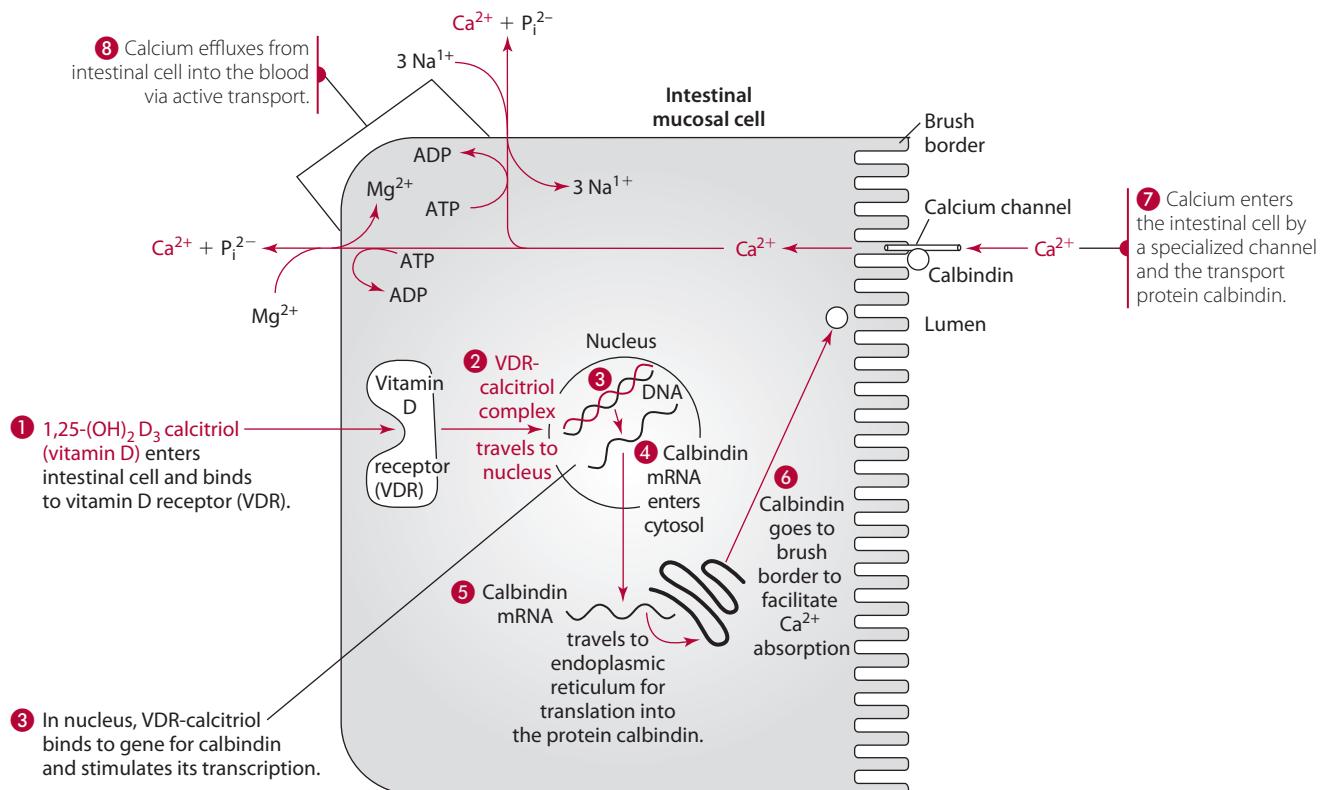


Figure 10.15 Vitamin D is needed for calcium absorption into intestinal cells.

cytosol and finally calcium release across the basolateral membrane by exocytosis.

With respect to phosphorus, calcitriol is thought to increase the activity of brush border alkaline phosphatase, which hydrolyzes phosphate ester bonds, thereby enhancing phosphorus absorption. Calcitriol is also thought to modulate the number of carriers available for sodium-dependent phosphorus absorption at the intestinal (especially jejunum and ileum) brush border membrane.

Calcitriol and the Kidney Calcitriol appears to be involved in parathyroid hormone-induced stimulation of calcium reabsorption in the distal renal tubule (Figure 10.14). Calbindin D28k, a larger form of the protein calbindin D9k found in the intestine, is thought to be synthesized in response to genomic effects of calcitriol and to play a role in renal calcium reabsorption. Thus, with high PTH concentrations (as occur when blood calcium concentrations are too low), vitamin D promotes reabsorption of calcium back into blood. Phosphorus excretion by the kidney is enhanced and can result in lower serum phosphorus concentrations.

Calcitriol, 24R,25-(OH)₂ D₃, and the Bone With respect to bone, PTH, alone or with calcitriol (which has been found to be produced directly within bone), directs the mobilization of calcium and phosphorus from bone to help achieve a normal blood calcium concentration (Figure 10.14). Interaction of PTH or calcitriol with receptors found on mature osteoblasts appears to induce the expression of receptor activator of NFκB ligand (RANKL). RANKL from osteoblasts interacts with the receptor protein RANK, found on the cell surface of immature monocytic osteoclast precursors (also called pre-osteoclasts) and stimulates the production and maturation of osteoclasts [7]. Osteoclasts in turn mobilize calcium and phosphorus from bone by releasing hydrochloric acid, alkaline phosphatase, collagenase, and other hydrolytic enzymes and substances, which dissolve and catabolize (eat away at) the bone matrix. The net effect of these actions is an increase in blood calcium and phosphorus concentrations at the expense of the bone.

Should blood calcium levels begin to rise above normal concentrations, calcitonin (a hormone produced by endocrine cells located in the connective tissue of the thyroid gland) is released and promotes the deposition (mineralization) of calcium and phosphorus in bones. Calcitriol or the metabolite 24R,25-(OH)₂ D₃ also may be involved in bone mineralization. Elevated serum calcitriol and elevated ionized serum calcium in turn cause a decrease in PTH production through feedback loops. The long feedback loop is indirect because of the elevated ionized serum calcium's inhibitory effect on PTH secretion. The short feedback loop is direct: The calcitriol decreases the transcription of the gene for parathyroid hormone, presumably by interacting with the vitamin D receptor in the parathyroid tissue and influencing the regulatory region of the PTH gene [10].

Cell Differentiation, Proliferation, and Growth

The local presence of the calcitriol (1,25-[OH]₂ D₃) within the tissues appears to regulate cell growth, differentiation, and proliferative activity in a variety of different tissues. For example, premalignant white blood cells and stem cells differentiate into macrophages and monocytes in the presence of adequate calcitriol. Calcitriol also induces cell differentiation of stem cell monocytes in the bone marrow to become mature osteoclasts.

Calcitriol also appears to decrease proliferation of some cells such as fibroblasts, keratinocytes, and lymphocytes. Vitamin D's ability to stimulate skin epidermal cell differentiation while preventing proliferation provides potential for treating many skin diseases. In fact, these functions of the vitamin have been applied in using vitamin D to treat psoriasis (a disorder marked by proliferation of the keratinocytes and a failure to differentiate rapidly). Vitamin D helps decrease proliferation associated with psoriasis and enhance differentiation of the epidermis.

Proliferation of abnormal intestinal, lymphatic, mammary, and skeletal cells (to name a few) is also diminished by vitamin D. For example, vitamin D appears to be able to down-regulate cancer cell growth in some tissues as well as being able to induce apoptosis if needed. Inadequate vitamin D status has been associated with an increased risk of developing and dying of many cancers, such as prostate, breast, colon, ovarian, and non-Hodgkin's lymphoma [7,13]. Potential uses of vitamin D and vitamin D analogs in treating of bone disease, hyperparathyroidism, cancer, and other conditions are under investigation.

Other Roles

Vitamin D is also purported to have a role in regulating blood pressure and preventing autoimmune disorders. The vitamin appears to down-regulate both renin and angiotensin production and thereby lower blood pressure [6]. Some autoimmune conditions including rheumatoid arthritis, Crohn's disease, multiple sclerosis, and type 1 diabetes mellitus, have been linked with inadequate vitamin D status. Studies have shown associations between serum 25-OH vitamin D concentrations and autoantibodies linked with diabetes. Moreover, protective effects against developing some of these diseases have been found with a daily intake of 50 µg (2,000 IU) vitamin D in children [7,14]. In addition, decreases (40%) in the risk of developing multiple sclerosis and rheumatoid arthritis with ingestion of 400 IU (10 µg) also have been reported [7].

Several immune-related functions appear to be influenced by vitamin D. Many of the immune system cells such as macrophages directly produce calcitriol. Some of the vitamin-mediated activities include cytokine production, lymphocyte and macrophage activity, and monocyte maturation [7]. Vitamin D, for example, appears to down-regulate production of some inflammatory cytokines interleukin (IL)2 and IL12 and to suppress the antigen-presenting

capacity of dendritic cells and macrophages [15]. Production of antimicrobial peptides, such as cathelicidin (needed to help fight the infective agent that causes tuberculosis), also is a function of adequate local calcitriol concentrations [16].

Vitamin D also appears to play an unclear role in pancreatic β -cell function for insulin secretion. Pancreatic β cell insulin secretion has been negatively related to serum 25-OH D₃ concentrations, whereas insulin sensitivity has been positively associated with serum 25-OH D₃ concentrations [15].

INTERACTIONS WITH OTHER NUTRIENTS

Discussion of vitamin D metabolism is impossible without noting the interrelationships among this vitamin/hormone and calcium, phosphorus, and vitamin K. The relationship with calcium and phosphorus is shown in Figure 10.14 and is discussed in the text corresponding to the figure. The interaction of calcitriol and vitamin K-dependent protein is discussed on page 413. Also speculated is a decrease in vitamin D absorption as a result of iron deficiency [17].

METABOLISM AND EXCRETION

Calcitriol hydroxylation at carbon 24 generates the metabolite 1,24,25-(OH)₃D₃ (Figure 10.16), which may be further oxidized to 1,25-(OH)₂ 24-oxo D₃. Subsequent reactions, including side-chain cleavage, yield calcitroic acid (Figure 10.16). Other vitamin D metabolites are also formed after hydroxylation and oxidation. These other vitamin D metabolites may be conjugated and then excreted primarily in the bile. Most vitamin D metabolites (more than 70%) are excreted in the feces, with lesser amounts excreted in the urine.

ADEQUATE INTAKE

Current recommendations for vitamin D are being widely criticized as inadequate. At the time of the last recommendations published in 1997, the requirement for vitamin D was unknown. Recommendations for

the vitamin suggested an Adequate Intake (AI) for vitamin D of 5 μ g or 200 IU daily for infants over 6 months, children, adolescents and for adults age 19 to 50 years, including women who are pregnant or lactating [7,18]. Newer studies suggest the requirement may be at least 500 IU (12.5 μ g) and that an intake $>1,000$ IU (25 μ g) vitamin D may be needed by some (especially those without sun exposure) to maintain (~ 80 nmol/L or 32 ng/mL) generally adequate 25-OH vitamin D concentrations [7,8,14,19,20]. This amount (1,000 IU) of the vitamin is thought to be obtainable by exposure to sunlight for about 5 to 15 minutes between about 10 A.M. and 3 P.M. during spring, summer, and fall [7]. In the continental United States, about 1.5 IU vitamin D/cm²/hour during the winter and about 6 IU/cm²/hour during the summer can be synthesized in the skin [21]. At higher latitudes and during winter months, the UVB photon path length is longer, resulting in less vitamin D production in the skin. In addition to season of the year, latitude, and time of day, in older people diminished organ function impairs vitamin D production. The published AIs for vitamin D for those age 51 to 70 years or >70 years are higher, 10 μ g (400 IU) and 15 μ g (600 IU), respectively [18]. However, as with recommendations for younger people, these AIs are thought to be inadequate. A vitamin D supplement providing 400 to 1,000 IU is likely needed for most elderly, particularly those who drink little milk and are partially or totally housebound.

DEFICIENCY: RICKETS AND OSTEOMALACIA

In infants and children, vitamin D deficiency results in **rickets**, which is characterized by seizures as well as growth retardation and failure of bone to mineralize. In vitamin D-deficient infants, epiphyseal cartilage continues to grow and enlarge without replacement by bone matrix and minerals. These effects are especially visible at the wrists, ankles, and knees, all of which enlarge. In addition, long bones of the legs bow and knees knock as weight-bearing activity such as walking begins. The spine becomes curved, and pelvic and thoracic deformities—such as rachitic rosary, characterized by costochondral beading at the juncture of the ribs and cartilages—occur.

In adults, deprivation of vitamin D leads to mineralization defects called **osteomalacia**. The defect results from changes in calcium and phosphorus absorption and excretion. For example, with vitamin D deficiency, less calcium is absorbed. Declining serum calcium concentrations trigger increased secretion of PTH. With continued poor calcium absorption caused by vitamin D deficiency, PTH remains elevated in the blood for prolonged periods. PTH promotes bone resorption and increased urinary phosphorus excretion, among other changes. Indicators of bone resorption include increased urinary excretion of bone collagen by-products such as hydroxyproline, N-telopeptide,

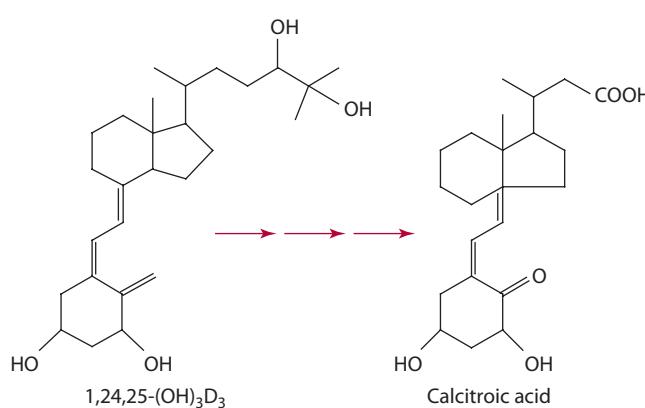


Figure 10.16 Some metabolites of vitamin D.

pyridinoline, and deoxypyridinoline. With an insufficient serum calcium \times phosphorus product, the mineralization of bones cannot occur. Thus, in vitamin D-deficient adults, as bone turnover occurs, the bone matrix is preserved, but remineralization is impaired. The bone matrix becomes progressively demineralized, producing radiographic changes and resulting in bone pain (characterized as throbbing or aching) and osteomalacia (soft bone).

Natural exposure to sunlight maintains adequate vitamin D nutrition for most of the world's population; however, people with insufficient sun exposure may be at risk for vitamin D deficiency. In addition to inadequate sun exposure, certain diseases, conditions, and populations may be risk factors for vitamin D deficiency. The elderly represent one population group that typically has insufficient vitamin D intake and low sunlight exposure. In addition, aging reduces synthesis of cholecalciferol in the skin and reduces the activity of renal 1-hydroxylase in response to PTH. Impaired vitamin D absorption may occur in disorders characterized by fat malabsorption, such as tropical sprue and Crohn's disease. Disorders affecting the parathyroid, liver, or kidney impair synthesis of the active form of the vitamin. People on anticonvulsant drug therapy may develop an impaired response to vitamin D and exhibit problems with calcium metabolism. Infants may be at risk for deficiency because human milk is low in vitamin D and infants' exposure to sunlight typically is minimal [22].

Vitamin D supplements are widely prescribed for people with renal disease, because their kidneys are typically unable to synthesize calcitriol needed for release into blood. Rocaltrol (Hoffman-LaRoche) is a commonly used oral calcitriol supplement. Calcijex (Abbott Laboratories) is given intravenously and often is used by people with kidney disease. Other preparations, such as Calderol (Organon-USA), which provides 25-OH D₃, also are available for use by people with organ malfunction. Deficiencies may be corrected with supplements of the vitamin—usually one 50,000 IU dosage given once per week for 8 weeks is sufficient [6,7].

TOXICITY

Although excessive exposure to sunlight may be the primary risk factor in developing skin cancer, it poses no risk of toxicity through overproduction of endogenous cholecalciferol. Extensive whole-body irradiation with ultraviolet light generally raises the level of circulating 25-OH D₃ to 100 to 200 nmol/L (40 to 80 ng/mL); levels greater than about 375 nmol/L (150 ng/mL) are associated with possible toxicity [7,9,18]. Photochemistry regulates the cutaneous production of vitamin D₃, thereby protecting people excessively exposed to sunlight from vitamin D intoxication.

Exogenous dietary ingestion of large amounts of vitamin D is one of the most likely of all vitamin excesses to cause overt toxic reactions. With excessive dietary ingestion of vitamin D, the vitamin is absorbed and incorporated into

chylomicrons, the remnants of which deliver the vitamin to the liver. There, the vitamin is hydroxylated in position 25 and released to the blood. Although the efficiency of 25-hydroxylase is decreased when the vitamin is present in abundance, the enzyme 25-hydroxylase is not well regulated, and thus an excessive amount of the metabolite can be produced with oversupplementation. Calcidiol in high concentrations may stimulate some of the same actions as calcitriol.

In the 1950s, an epidemic of "idiopathic hypercalcemia" among English infants was traced to an intake of vitamin D between 2,000 and 3,000 IU per day. Symptoms of toxicity in the infants included anorexia, nausea, vomiting, hypertension, renal insufficiency, and failure to thrive. Eight people experienced hypervitaminosis from consuming milk (1/2 to 3 cups daily from a local dairy) that contained up to 232,565 IU of vitamin D₃ per quart; they displayed hypercalcemia and hypercalcidiolemia [23]. Dosages of 10,000 IU per day for several months have resulted in hypercalcemia and associated calcification of soft tissues (calcinosis) such as the kidney, heart, lungs, and blood vessels, as well as hyperphosphatemia, hypertension, anorexia, nausea, weakness, renal dysfunction (characterized by polyuria, polydipsia, azotemia, nephrolithiasis, and renal failure), and, in some cases, death [17,24]. The tolerable upper intake level is set at 50 µg (2,000 IU) per day [18], but has been criticized as being too low [16,19,20]. A limit of 250 µg (10,000 IU) per day has been proposed based on risk assessment [25].

ASSESSMENT OF NUTRITION

The plasma concentration of 25-OH D₃ (calcidiol) is often used as an index of vitamin D status. Concentrations <~37 nmol/L (15 ng/mL) are associated with subclinical deficiency, and concentrations <28 nmol/L (11 ng/mL) suggest vitamin D deficiency. Newer findings report insufficient vitamin D status with circulating 25-OH D₃ concentrations of ≤80 nmol/L (32 ng/mL); a concentration of 80 nmol/L is associated with a plateau in PTH concentrations [7,13]. Optimal serum 25-OH D₃ concentrations are suggested as 80–120 nmol/L or about 30–60 ng/mL [14,26]. Toxicity is considered when serum 25-OH D₃ concentrations typically exceed 375 nmol/L [7,9,18,25].

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Vitamin E

Vitamin E typically has encompassed eight compounds (vitamers). Each of these eight compounds contains a phenolic functional group on a chromanol/chromane

ring (sometimes called the head of the molecule) and an attached phytol side chain (sometimes called the **phytol tail** of the molecule). The eight compounds (Figure 10.17) are usually divided into two classes:

- the tocopherols, which have saturated side chains with 16 carbons
- the tocotrienols (also called trienols), which have unsaturated side chains with 16 carbons

Each class is composed of four vitamers that differ in the number and location of methyl groups on the chromanol ring. Vitamers in both classes are designated as α , β , γ , or δ . Only α -tocopherol has biologic activity. All tocopherols and tocotrienols found naturally in foods have an RRR stereochemistry. R and S are used to designate stereoisomers of asymmetrical molecules such as vitamin E. The most biologically active form is thus RRR α -tocopherol, which was once called d- α -tocopherol.

Synthetic ester forms of α -tocopherol include all racemic (all-rac) α -tocopheryl acetate and all-rac α -tocopheryl succinate, which are used in vitamin supplements and fortified foods. These synthetic forms of the vitamin often contain a mixture of eight stereoisomers and thus are not as active as the naturally occurring form, RRR α -tocopherol. Of the eight stereoisomers, four are in the 2R-stereoisomeric form (RRR, RSR, RRS, and RSS) and four are in the 2S-stereoisomeric form (SRR, SSR, SRS, and SSS) [1]. The form of the vitamin should be listed on the food or supplement label.

In the report on vitamin E by the Food and Nutrition Board to establish recommended intakes, vitamin E activity is limited to naturally occurring RRR α -tocopherol and to three synthetic stereoisomeric forms (RSR, RRS, and RSS) of α -tocopherol [1]. In establishing a tolerable upper intake level, all supplemental forms of the vitamin are considered [1].

The term *tocopherol* is derived from the Greek word *tokos*, which means “childbirth,” and *phero*, which means “to bear or bring forth.” This terminology is based on the vitamin’s discovery by H. Evans and K. Bishop back in the early 1920s, when they found that rats could not reproduce when given a diet of rancid lard. Wheat germ oil provided the needed vitamin; the oil was later purified, and the vitamin was extracted and named vitamin E (following D which had been previously discovered), by Emerson.

SOURCES

Vitamin E, in its various forms, is found in both plant and animal foods. Plant foods, especially oils from plants, are considered the richest and main sources of vitamin E. Oils high in α -tocopherol include canola, olive, sunflower, safflower, and cottonseed. Soybean and corn oils contain some α -tocopherol but considerably higher amounts of γ -tocopherol [2,3]. Foods (especially full-fat varieties)

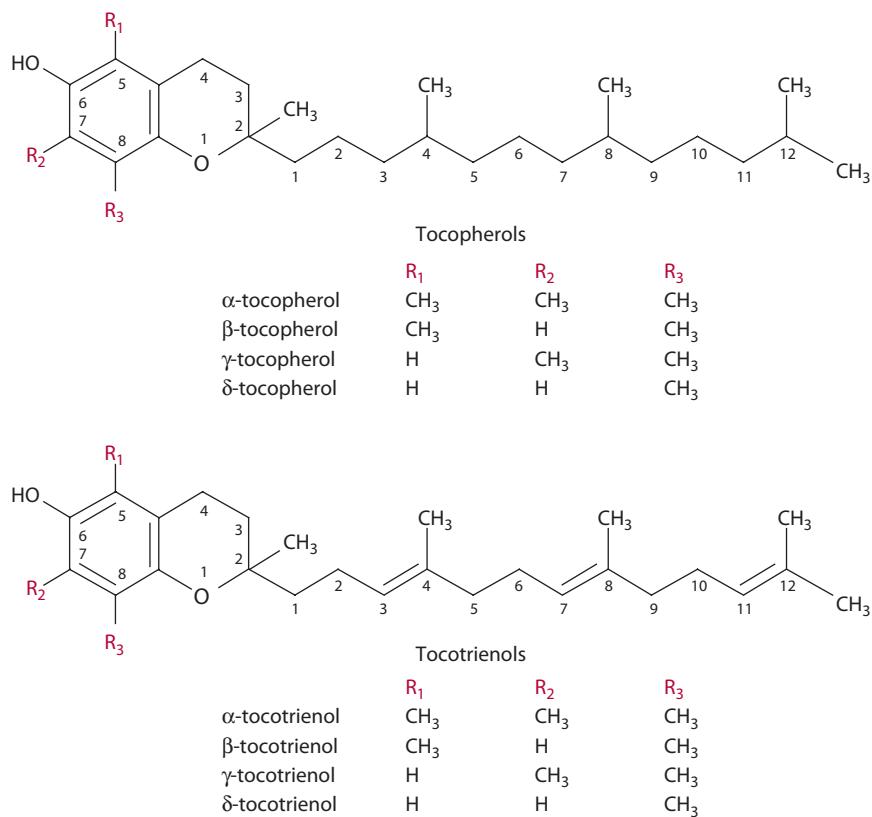


Figure 10.17 The structures of the various forms of the tocopherols and tocotrienols.

made from vegetable oils, such as salad dressings, mayonnaise, and margarine, and foods made from nuts, such as peanut butter, represent good sources of vitamin E [2,3]. Unfortunately, people limiting fat intake also limit foods that are high in vitamin E and thus may compromise their ability to meet dietary intake recommendations for the vitamin. Other plant sources of vitamin E include whole-grain cereals, legumes, and some fruits and vegetables. The leaves and other green (chloroplast) portions of plants contain mostly α -tocopherol with small amounts of γ -tocopherol. The main sources of γ , δ -, and β -tocopherols are nonchloroplast regions of plants. Tocotrienols are found in legumes and cereal grains such as wheat, barley, rice, and oats [2,3]. The bran and germ sections of cereals are especially rich in tocotrienols. Thus, wheat germ oil and wheat bran represent significant sources of tocotrienols.

In foods of animal origin, vitamin E, primarily α -tocopherol, is found concentrated in fatty tissues of the animal. Thus, higher-fat meats can provide some vitamin E. However, compared to plants, animal products represent an inferior source of vitamin E. Table 10.3 lists the approximate α -tocopherol equivalents found in commonly consumed foods. The contributions of the four tocopherols and the four tocotrienols are included in α -tocopherol equivalents, with adjustments for bioavailability. Before the 2000 Dietary Reference Intakes publication with recommendations for vitamin E [1], the vitamin E content of foods

and recommendations for the vitamin were expressed as α -tocopherol equivalents. Most food composition tables report the vitamin E content of foods as α -tocopherol equivalents, but composition tables providing the actual α -tocopherol content of foods are being developed. Calculations to directly convert α -tocopherol equivalents to α -tocopherol for food are not possible, but an analysis of the dietary intake data from the National Health and Nutrition Examination

Table 10.3 Approximate Vitamin E Content of Foods as α -Tocopherol Equivalents

Food	mg/100 g
Oils:	
Wheat germ	192
Corn	21
Cottonseed	38
Peanut	13
Safflower	43
Soybean	18
Sunflower	51
Margarine	15
Nuts	0.69–9
Breads	0.4
Vegetables	0.1–2.0
Fruits	0.1–1.1
Meat, fish	1
Eggs	1

Source: www.nal.usda.gov/fnic/foodcomp

Survey III revealed that about 80% of total dietary vitamin E was in the form of α -tocopherol [1].

Vitamin E, like other fat-soluble vitamins, is susceptible to destruction during food preparation, processing, and storage. Tocopherols can be oxidized with lengthy exposure to air. In addition, exposure of the vitamin to light and heat also can lead to increased destruction.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Whereas the tocopherols are found free in foods, the tocotrienols are found esterified and must be hydrolyzed before absorption. Similarly, synthetic ester forms of the tocopherols such as tocopheryl acetate must be digested before absorption. Pancreatic esterase and especially duodenal mucosal esterase (also called carboxyl ester hydroxylase) are thought to function in the lumen or at the brush border of the intestine to hydrolyze tocotrienols and synthetic ester α -tocopherols for absorption [4].

Vitamin E is absorbed primarily in the jejunum by non-saturable, passive (requiring no carrier) diffusion. Bile salts are required for emulsification, solubilization, and micelle formation allowing the vitamin to diffuse across enterocyte membrane. Simultaneous digestion and absorption of dietary lipids with vitamin E improve the absorption of vitamin E; however, the optimum amount of fat needed to improve absorption has not been identified [1]. In addition, the extent of absorption of vitamin E is unclear, with results of studies ranging from 20% up to about 80% [5,6]. Similar efficiency of absorption has been demonstrated among RRR and SRR α -tocopherols and RRR all-rac α -tocopherols [7–10].

In the enterocyte, absorbed tocopherol is incorporated into chylomicrons for transport through the lymph into circulation. During tocopherol transport in the chylomicrons, tocopherol equilibrates or is transferred among the plasma lipoproteins, including HDLs and LDLs, which contain the highest concentrations of the vitamin [11]. LDLs are thought to contain about 5–9 α -tocopherol molecules per LDL [12]. Tocotrienols also are found in the same lipoproteins, but in lower concentrations than α -tocopherol. The half-life for RRR α -tocopherol in humans is about 48 hours, and the stereoisomer SRR α -tocopherol has a half-life of about 13 hours [13].

Chylomicron remnants deliver vitamin E (absorbed tocopherols and tocotrienols) to the liver. Only RRR α -tocopherol appears to be incorporated into very low density lipoproteins (VLDLs) for resecretion back into the blood and transport to other tissues. A specific protein, called α -tocopherol transfer protein (α TTP), which is made in the liver, appears to be necessary for the transfer of tocopherol (RRR α -tocopherol preferentially) into VLDLs, which enable distribution of the vitamin to tissues. A deficiency or absence of α TTP caused by gene defects leads

to a vitamin E deficiency syndrome. Because of the specificity of the transfer protein, other forms of the vitamin are poorly recognized by the protein and are not resecreted into the circulation by VLDLs.

Tocopherol uptake into cells occurs as lipoproteins are taken up by body tissues. Thus, uptake of the vitamin can occur in several ways:

- as receptor-mediated uptake of LDLs occurs
- through lipoprotein lipase-mediated hydrolysis of chylomicrons and VLDLs
- through HDL-mediated nutrient delivery
- possibly by other mechanisms

A phospholipid transfer protein also is thought to facilitate vitamin E transfer from lipoproteins to membranes [14].

Within the cell cytoplasm as well as other parts of the cell, including the nucleus, vitamin E appears to bind to specific proteins (tocopherol-binding proteins) for transport. One protein that has been identified and thought to be involved in cellular trafficking and efflux of the vitamin from cells is the transporter adenosine triphosphate–binding cassette (ABC) A1. The protein is also known to transport cholesterol and phospholipids.

Vitamin E is found within the cell primarily in cell membranes such as the plasma, mitochondrial, and microsomal membranes. Vitamin E's chromanol group likely is directed toward the membrane surface (near the phosphate region of the phospholipid), and its phytyl tail is directed near the hydrocarbon region [15].

There is no single storage organ for vitamin E. The largest amount (over 90%) of the vitamin is concentrated in an unesterified form in fat droplets in the adipose tissue, with smaller amounts in liver, lung, heart, muscle, adrenal glands, spleen, and brain. The concentration of vitamin E in adipose tissues increases linearly with dosage of vitamin E, whereas vitamin E concentration in the other tissues remains constant or increases only at a very slow rate [16]. Release of vitamin E from adipose tissues, however, is slow even during periods of low vitamin E intake. The liver and plasma concentrations of the vitamin provide a readily available source. Vitamin E from other storage sites, such as the heart and muscle, can be used at intermediate rates [15].

FUNCTIONS AND MECHANISMS OF ACTION

The principal function of vitamin E is the maintenance of membrane integrity, including possible physical stability, in body cells. The mechanism by which vitamin E protects the membranes from destruction is through its ability to prevent the oxidation (peroxidation) of unsaturated fatty acids contained in the phospholipids of the membranes. The phospholipids of the mitochondrial membrane and endoplasmic reticulum contain more unsaturated fatty acids than the cell's plasma membrane and thus are at

greater risk of oxidation. However, cell membranes are still vulnerable to oxidation. Tissues with cell membranes especially susceptible to oxidation include the lungs, brain, and erythrocytes. Erythrocyte membranes, for example, are high in polyunsaturated fatty acids and are exposed to high concentrations of oxygen. Because it prevents oxidation, vitamin E is considered an antioxidant. A discussion of vitamin E's role as an antioxidant follows, with a brief description of the generation of carbon-centered and peroxy radicals. More information on how free radicals are generated and how they can damage cell membranes may be found in this chapter's Perspective, "The Antioxidant Nutrients, Reactive Species, and Disease."

Antioxidant Role

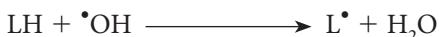
As an antioxidant, vitamin E, especially α -tocopherol can stop reactions involving free radicals (free radical termination) and can destroy singlet molecular oxygen. This section addresses each of these aspects of vitamin E function.

Free Radical Termination The structure of vitamin E, specifically its phenolic ring, enables hydrogen ions to be donated to free radicals. Of the different forms of the vitamin, α -tocopherol is more effective than β -, γ -, or δ -tocopherol in its ability to donate hydrogen atoms. The hydrogen ions from α -tocopherol effectively and quickly react with and terminate a variety of free radicals before the free radicals can destroy cell membranes and other cell components.

Free radicals are generated in the course of many body processes involving enzymatic reactions or with exposure to ultraviolet light, among other events. Free radicals can start a series of reactions that can be terminated by vitamin E. The reactions occur in three phases: initiation, **propagation** (ongoing generation), and termination, with the last involving vitamin E. A description of the three phases, including the reactions occurring in each, is presented next.

Initiation typically begins with an initiator such as a free radical. For example, hydroxyl radicals ($\cdot\text{OH}$) are very highly reactive, rapidly taking electrons from the surroundings. Often the electron taken by the reactive free hydroxyl radical is from nearby organic molecules. If the organic molecule is a polyunsaturated fatty acid (PUFA) present in the phospholipid portion of the cell membrane, the membrane is damaged. Membrane lipid peroxidation is thought to represent a primary event in oxidative cellular damage. Specifically, hydrogen atoms from the methylene groups ($-\text{CH}_2-$) found between double bonds in polyunsaturated fatty acids ($-\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}-$) are primary targets for proton abstraction by radicals.

- The reaction between lipid compounds (LH) such as PUFA and free hydroxyl radicals ($\cdot\text{OH}$) leads to the formation of a lipid carbon-centered or alkyl radical (L^\bullet) and water, as shown here and in Figure 10.18:



- Alternately, lipid compounds (LH) can react with molecular oxygen (O_2) to generate lipid carbon centered or alkyl radicals and the **hydroperoxyl radical** HO_2^\bullet , as follows:



Once lipid carbon-centered or alkyl radicals are formed, they may react to form additional radicals in propagation reactions.

Propagation is the second step in the process of lipid peroxidation.

- Lipid carbon-centered or alkyl radicals can react with molecular oxygen in a propagation reaction to form lipid peroxy radicals, LOO^\bullet and promote peroxidation, as shown here and in Figure 10.18:



Peroxy radicals (LOO^\bullet), once formed, can abstract a hydrogen atom from other organic compounds including more polyunsaturated fatty acids (L'H) in membranes or in lipoproteins to generate lipid hydroperoxides (LOOH) and a chain reaction with the L^\bullet , as shown here and in Figure 10.18:



Termination is the final step. Chain reactions involving L^\bullet must be terminated to minimize cellular damage. Preventing damage from oxygen radicals depends on a complex protective system, of which vitamin E is a part.

Vitamin E located in or near membrane surfaces can react with peroxy radicals (LOO^\bullet) before they interact with fatty acids in cell membranes or other cell components. Thus, vitamin E terminates chain-propagation reactions. Vitamin E is less effective, however, in terminating peroxidation that generates free hydroxyl radicals ($\cdot\text{OH}$) or alkoxyl radicals (RO^\bullet).

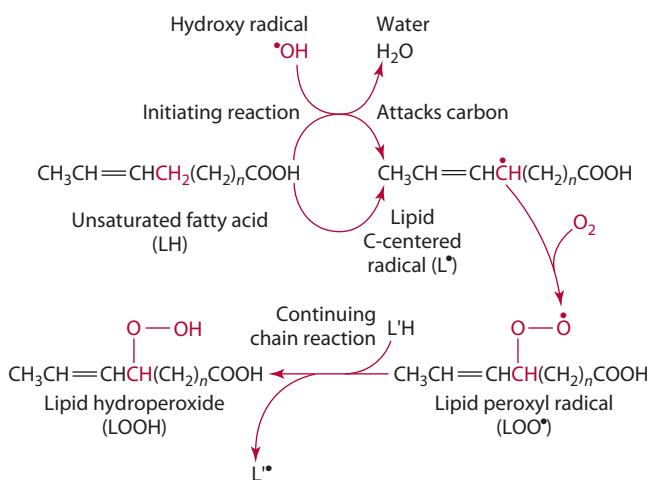


Figure 10.18 Initiating and chain reactions caused by hydroxyl radical attack on unsaturated fatty acid.

Vitamin E (EH, reduced state), because of the reactivity of the phenolic hydrogen on its carbon 6 hydroxyl group and the ability of the chromanol ring system to stabilize an unpaired electron, can provide a hydrogen for the reduction of peroxy radical, as shown:



Vitamin E (EH) also provides a hydrogen for the reduction of lipid carbon-centered radicals, as shown:



E^\bullet represents oxidized vitamin E (also called an α -tocopherol radical or a tocopheroxyl radical). The process is sometimes called “free-radical scavenging.” Termination is achieved when two free radicals combine to form a molecule that is not a free radical and cannot continue the reaction.

The tocopheroxyl radical that is generated in the termination must be reduced to be reused. Regeneration of reduced vitamin E (Figure 10.19) requires reducing agents, which include vitamin C (ascorbic acid), reduced glutathione (GSH), NADPH, ubiquinol, and dihydrolipoic acid [17]. In addition, tocopheroxyl radicals can react with another peroxy radical to form inactive products such as tocopherylquinone.

Vitamin E is only one line of defense against oxidative tissue damage. Other parts of the protection include vitamin C, glutathione, carotenoids, and enzymes that require a variety of trace or microminerals (iron, selenium, zinc, copper, and manganese) for their activation. Therefore, an interrelationship exists among vitamins E and C, carotenoids, and these minerals involved in antioxidant activities. Vitamins C and E appear to work synergistically in inhibiting oxidation. The relationship between vitamin E and other nutrients with antioxidant functions is reviewed in the Perspective at the end of this chapter.

Singlet Molecular Oxygen Destruction Singlet molecular oxygen, ${}^1\text{O}_2$, generated from lipid peroxidation of membranes, transfer of energy from light (photochemical reactions), or the respiratory burst occurring in neutrophils (enzymatic reactions), for example, is another very reactive and destructive compound that may be formed in the body. Singlet molecular oxygen readily reacts with organic molecules such as protein, lipids, and DNA and thus can damage cellular components unless removed. Quenching

is a process by which electronically excited molecules, such as singlet molecular oxygen, are inactivated. Specifically, physical quenching occurs when the singlet excited oxygen is deactivated without light emission and generally involves electron energy transfer [18]. This process was discussed earlier in this chapter in the section on the antioxidant functions of carotenoids (page 386). However, carotenoids are not alone in their ability to quench singlet oxygen. Vitamin E also has oxygen-quenching abilities. The ability of vitamin E to physically quench singlet oxygen is related to the free hydroxyl group in position 6 of vitamin E's chromane ring (Figure 10.17). Yet all tocopherols are not equal in their quenching abilities: α -tocopherol was found to be as or more effective in the quenching of singlet molecular oxygen than β -tocopherol, followed in descending order by γ -tocopherol and then δ -tocopherol [18]. Moreover, the ${}^1\text{O}_2$ -quenching ability of the carotenoids lycopene and β -carotene is about two orders of magnitude greater than that of vitamin E; however, given the lower plasma concentrations of the carotenoids, vitamin E's role in quenching singlet oxygen is of physiological significance [18].

Other Roles

Other, nonantioxidant roles of vitamin E have been demonstrated. Tocotrienols, for example, appear to affect cholesterol metabolism. Suppression of the activity of the rate-limiting enzyme 3-hydroxy-3-methyl-glutaryl (HMG) CoA reductase in cholesterol synthesis by tocotrienol has been shown in vitro [19]. These findings are consistent with observations that tocotrienols reduce plasma cholesterol concentrations in animals and in humans [20].

Suppression of tumor growth and cell proliferation also has been attributed to tocotrienols, although in general, diets high in vitamin E have not been associated with lower risk of cancers [21–24]. Protein kinase C, important for signal transduction and cell growth and differentiation, may be inhibited by α -tocopherol [24]. In addition to links with cancer, vitamin E also has been associated with other conditions as reviewed hereafter.

Vitamin E and Heart Disease Clinical trials with vitamin E alone as well as with other antioxidants suggest that the vitamin may decrease the susceptibility of LDL to oxidation by free radicals. High vitamin E intake has been associated with a lower risk of coronary heart disease in large cohort studies involving women [25] and men [26].

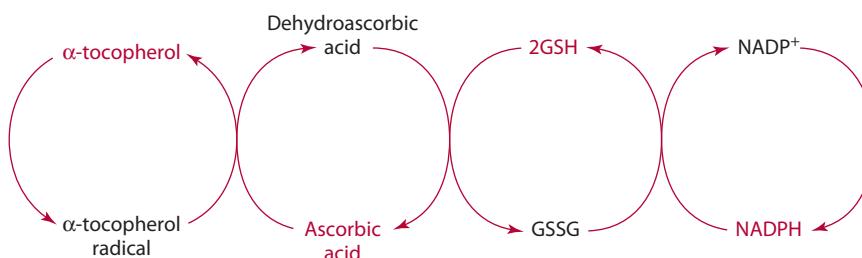


Figure 10.19 The regeneration of vitamin E (α -tocopherol).

Supplementation with 800 IU vitamin E, 1 g vitamin C, and 24 mg β -carotene significantly reduced the susceptibility of LDL to oxidation in patients with cardiovascular disease [27]. Supplementation with α -tocopherol (800 IU) alone was found to be as effective as a combination of ascorbate (1 g), β -carotene (30 mg), and α -tocopherol (800 IU) in decreasing LDL oxidation [28]. Supplementation with 400 or 800 IU (268 or 567 mg) α -tocopherol in another group of patients with confirmed heart disease reduced the rate of both nonfatal and total heart attacks [29].

These findings showing decreased oxidation have implications for preventing atherosclerosis. Briefly, atherosclerosis is thought to begin with an accumulation of lipid-laden foam cells in the arterial intima. Radical-induced oxidation of apoprotein B100, for example, in LDL is thought to be involved in promoting scavenger receptor-mediated uptake of the LDL by macrophages. Macrophages, which develop into foam cells, are thought to take up oxidized LDL more readily than nonoxidized LDL. With continued accumulation, fatty streaks develop and represent the initial steps in atherosclerosis. Oxidized LDL may also reduce macrophage motility in the arterial intima, increase monocyte accumulation in endothelial cells, and increase cytotoxicity of endothelial cells to contribute to atherogenicity [25,26]. Thus, vitamin E's ability to prevent or decrease LDL oxidation thwarts the development of atherosclerotic lesions.

Several studies completed over the past five or so years, however, have not shown beneficial effects of vitamin E supplementation. For example, clinical trials providing vitamin E (400 IU or 300 mg) to people who had either a heart attack or diagnosed heart disease showed no beneficial effects in decreasing risk of death [30,31]. The Heart Outcomes Prevention Evaluation (HOPE) study found no difference in rates of death from heart attack, stroke, or heart-related conditions in over 7,000 men and women with heart disease, peripheral vascular disease, a previous stroke, or diabetes who took 400 IU α -tocopherol for 7 years, compared with rates in a placebo group [32]. Interestingly, 5.8% of participants taking the vitamin E were hospitalized with heart failure, compared with 4.2% of participants taking the placebo. In a meta-analysis of 19 randomized trials of vitamin E, which included about 136,000 people, a dose-response relationship between vitamin E and all-cause mortality was reported in those who took at least 400 IU vitamin E per day for at least one year [33]. Trials that compared 600 mg vitamin E, 250 mg vitamin C, and 20 mg β -carotene with a placebo failed to show any significant reductions in 5-year mortality, or incidence of any type of vascular disease, cancer, or other major outcome in a group of 20,536 adults with heart disease or diabetes in the United Kingdom [34]. Postmenopausal women with heart disease ($n = 423$) randomized to receive 400 IU vitamin E and 500 mg vitamin C twice daily had significantly higher total and cardiovascular mortality than those receiving a placebo [35].

Vitamin E and Eye Health Vitamin E has been suggested for treating or preventing other disorders. Cataracts result in part from oxidative damage to proteins that then aggregate and precipitate in the lens to cause lens opacities or cloudiness. Oxygen and oxyradicals are thought to contribute to the development of cataracts. Poor antioxidant status or intake, especially of vitamins E and C, has been shown in many but not all studies to be associated with development of cataracts and age-related macular degeneration [36–46]. Although some epidemiological studies suggest a protective effect of vitamin E against these conditions, the effect cannot be attributed solely to vitamin E, because subjects in most of the studies were consuming a multivitamin preparation [38–46].

Other Conditions Vitamin E has also been suggested for people with conditions characterized by increased lipid peroxidation. For example, iron toxicity typically leads to increased lipid peroxidation through production of free radicals and causes excessive damage to organs, especially the liver [47]. People with diabetes mellitus also experience increased peroxidation. Vitamin E supplementation (600–900 mg α -tocopherol) by people with type 2 diabetes mellitus decreased oxidative damage and improved metabolic control [48–50]. Vitamin E was thought to perhaps improve plasma membrane structure and its related activities required for glucose transport and metabolism (and thus metabolic control) [48–50]. By diminishing lipid peroxidation and increasing GSH availability, vitamin E could help maintain cell membrane fluidity, which in turn may improve glucose transporter function and thus insulin-dependent cellular glucose uptake [51]. Anti-inflammatory effects also have been observed with vitamin E supplementation [52].

INTERACTIONS WITH OTHER NUTRIENTS

Because the antioxidant functions of vitamin E in the body are closely tied to those of selenium-dependent glutathione peroxidase (an enzyme that converts lipid peroxides into lipid alcohols), an interrelationship exists between vitamin E and selenium. The actions of both nutrients are complementary, and higher concentrations of one nutrient can reduce the effects of lower concentrations of the other nutrient. Similarly, some of vitamin C's functions also complement vitamin E, and vitamin C can regenerate vitamin E following its oxidation.

To a lesser extent, interrelationships exist between vitamin E and sulfur-containing amino acids (S-aa). Cysteine, an S-aa generated from another S-aa, methionine, is necessary to synthesize glutathione, which serves as the reducing agent in the glutathione peroxidase reaction and regenerates (reduces) vitamin E if it has undergone oxidation.

The relationship between vitamin E and dietary polyunsaturated fatty acids has been suggested because the

requirement for the vitamin increases or decreases as the degree of unsaturation of fatty acids in body tissues rises or falls; body tissue lipids, in turn, are influenced by dietary lipid intake [1]. However, foods high in polyunsaturated fatty acids also tend to be relatively good sources of vitamin E.

High intake of vitamin E can interfere with several aspects of the other fat-soluble vitamins. Vitamin E inhibits β -carotene absorption and its conversion to retinol in the intestine [53–55]. Vitamin E may also impair vitamin K absorption [15,16,56,57]. In the vitamin K cycle, vitamin E or α -tocopherol quinone may block regeneration of the reduced form of vitamin K [15,16,56,57].

METABOLISM AND EXCRETION

Initial oxidation of α -tocopherol generates a tocopheroyl chromanoxyl radical, which may be reduced by vitamin C or glutathione, among other compounds, back to α -tocopherol or may be further oxidized to tocopherol quinone. In the liver, the tocopherol quinone may be reduced by α -tocopherol quinone reductase, using NADPH, to tocopherol hydroquinone. The side chain of tocopherol hydroquinone may be oxidized to form α -tocopheronic acid. Tocopheronic acid typically is conjugated with glucuronic acid and excreted in the urine. Another urinary metabolite, α -tocopheronolactone, also has been identified.

Oxidation of the phytol side chain of unoxidized α -tocopherol generates 2,5,7,8-tetramethyl 2-(2'-carboxyethyl) 6-hydroxychroman (abbreviated α -CEHC), which may be conjugated with glucuronic acid and excreted in the urine. Similarly, oxidation of the phytol side chain of γ -tocopherol produces 2,7,8-trimethyl 2-(2'-carboxyethyl) 6-hydroxychroman (abbreviated γ -CEHC), which also is typically conjugated with glucuronate and then excreted in the urine [58].

The major route of excretion for absorbed α -tocopherol is through the bile into the feces. An ABC transporter protein called MDR2 is thought to be involved in the excretion of the vitamin into the bile. In addition, fecal concentrations of the vitamin and its metabolites are relatively higher than urinary concentrations, because relatively little vitamin E is absorbed, and many of the vitamin forms (such as γ -, δ -, and β -tocopherols and tocotrienols) that are absorbed are not used. For example, γ -tocopherol is preferentially metabolized by the liver and excreted. Most of the vitamin E secreted into the bile for elimination is usually conjugated with glucuronic acid before excretion. Sebaceous glands in the skin also secrete vitamin E; this avenue may represent another minor means of excreting the vitamin.

RECOMMENDED DIETARY ALLOWANCE

The latest (2000) recommendations for the intake of vitamin E differ from those published in 1989 not only in the amount recommended but also in the form of the vitamin [1]. Previous recommendations for vitamin E included

the eight naturally occurring forms of the vitamin, but the newest recommendations are only for α -tocopherol. The rationale for this change is the preferential hepatic secretion and metabolism of RRR α -tocopherol. The units of intake for the vitamin have changed from mg α -tocopherol equivalents to mg α -tocopherol. The former units accounted for all eight naturally occurring forms of vitamin E with adjustments for bioavailability.

The RDA for vitamin E for adult men and women (including during pregnancy for women) is 15 mg α -tocopherol [1]. During lactation, women require slightly higher vitamin E intake, with an RDA of 19 mg [1]. The RDA for vitamin E for adults is based on the vitamin E requirement plus twice the coefficient of variation, rounded to the nearest mg [1]. People who smoke may have higher requirements for vitamin E, but specific recommendations for this population have not been made [59]. Recommendations for children age 9 to 13 and 14 to 18 years are 11 and 15 mg, respectively; those for children age 1 to 3 and 4 to 8 years are 6 and 7 mg, respectively [1]. Only an adequate intake (AI) for vitamin E was established for infants. The AI, RDA, and requirement for vitamin E are based on intake of the natural form (RRR) of α -tocopherol and the synthetic all-rac 2R-stereoisomeric forms (RSR, RRS, and RSS) of α -tocopherol used in fortified foods and vitamin supplements [1]. 1,500 IU RRR α -tocopherol is equivalent to 1,000 mg α -tocopherol. To estimate mg all-rac (synthetic) 2R α -tocopherol in a supplement, multiply the dose by 0.45 mg/IU, and to estimate mg RRR (natural) α -tocopherol in a supplement multiply the dose by 0.67 mg/IU [60]. Some have suggested that higher vitamin E recommendations are needed to achieve serum α -tocopherol concentrations of 13–14 mg/L (30–33 μ M/L); these serum concentrations (achieved with 100 IU vitamin E supplements) are thought to be associated with reduced mortality from chronic disease [60].

DEFICIENCY

A deficiency of vitamin E in humans is quite rare. Only a few population groups are at risk for deficiency, including those with fat malabsorption disorders such as cystic fibrosis (characterized by pancreatic lipase deficiency) and hepatobiliary system disorders, particularly chronic cholestasis (characterized by decreased bile production). A second group at risk are those with genetic defects in either lipoproteins or the α -tocopherol transfer protein [9, 61]. For example, abetalipoproteinemia is a rare genetic disease that may result in vitamin E deficiency because of a lack of microsomal transfer protein needed to assemble or secrete lipoproteins containing apolipoprotein B.

Some symptoms of vitamin E deficiency are skeletal muscle pain (myopathy) and weakness, ceroid pigment accumulation, hemolytic anemia, and degenerative neurological problems, including peripheral neuropathy,

cerebellar ataxia, loss of vibratory sense, and loss of coordination of limbs [5,53]. Plasma concentrations of total tocopherol relative to total lipids in adults decrease to <5 µg/mL or <0.8 mg/g with vitamin E deficiency [62,63].

TOXICITY

Vitamin E appears to be one of the least toxic of the vitamins [64]. Nonetheless, because of an increased tendency for bleeding, a tolerable upper intake level of 1,000 mg α-tocopherol (1,500 IU RRR α-tocopherol) for adults has been established by the Food and Nutrition Board [1]. This recommendation for an upper level of intake includes any form of supplemental α-tocopherol [1]. In addition to increased bleeding, higher intake of the vitamin also has been associated with gastrointestinal distress, including nausea, diarrhea, and flatulence; impaired blood coagulation; possible increased severity of respiratory infections, and occasional reports of muscle weakness, fatigue, and double vision [16,56,64–66]. Clinical trials with doses of the vitamin in excess of current recommendations are being conducted to examine vitamin E's effectiveness in preventing and treating various diseases. Findings may ultimately lead to higher recommended intake of the vitamin for specific populations with or at risk for a particular condition [1]. Some researchers advocate a tolerable upper intake level of 1,600 IU, which is equivalent to 1,070 mg RRR α-tocopherol [67].

ASSESSMENT OF NUTRITUDE

Accurately evaluating vitamin E status in humans remains difficult with current techniques. Normal serum vitamin E concentrations range from about 5 to 20 µg/mL in adults, and values <5 µg/mL indicate deficiency. In vitamin E deficiency, plasma α-tocopherol concentrations correlate with vitamin E intake [68]. Thus, plasma concentrations are responsive to dietary intake under limited conditions. In contrast, concentrations of the vitamin plateau with daily intake of at least 200 mg RRR or all-rac α-tocopherol [10].

A crude estimate of vitamin E status can be obtained from an erythrocyte hemolysis test that compares the amount of hemoglobin released by red cells during incubation with dilute hydrogen peroxide with the amount released during distilled water incubation. The result is expressed as a percentage, with >20% indicating deficiency [15]. Concentrations of α-tocopherol of 6 µg/mL are usually sufficient to prevent hemolysis; however, variables other than vitamin E status influence in vitro hemolysis [69].

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Vitamin K

Compounds with vitamin K activity all have a 2-methyl 1,4-naphthoquinone ring. The naturally occurring forms of vitamin K are phylloquinone (2-methyl 3-phytol 1,4-naphthoquinone), isolated from green plants, and menaquinones, which generally are synthesized by bacteria. Most of the menaquinones (abbreviated MK) contain about 6 to 13 **isoprenoid** units (often written as MK-6–13) in a side chain attached at the number 3 carbon.

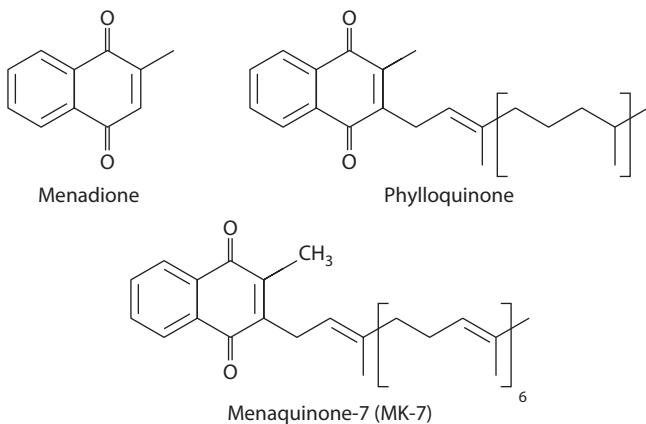


Figure 10.20 Biologically active forms of vitamin K.

Phylloquinone and menaquinone formerly were designated K1 and K2, respectively. Although most menaquinones are generated by bacteria, one is made from menadione. Menadione, 2-methyl 1,4-naphthoquinone (formerly called K3), is not found naturally but rather is a synthetic form of vitamin K that must be alkylated in the body by tissue enzymes for activity. Alkylation of menadione can generate MK-4. Figure 10.20 depicts menadione, phylloquinone, and one of the menaquinones, specifically menaquinone-7 (MK-7), which has seven isoprenoic units.

Vitamin K was named from the Danish word *koagulation*, which means “coagulation.” Back in the 1920s, H. Dam discovered that chicks fed a low-fat and cholesterol-free diet became hemorrhagic (that is, they bled excessively) and that their blood took a long time to clot. The missing vitamin called K that corrected the problem was identified in the early 1940s. Dam (along with Doisy) was recognized with a Nobel prize in medicine in 1941 for the discovery.

SOURCES

Dietary vitamin K is provided mostly as phylloquinone in plant foods and as a mixture of menaquinones in animal products. Bacteria in the gastrointestinal tract, especially the colon, provide a source of menaquinones for humans. Phylloquinone is thought to provide the majority of the vitamin in the U.S. diet [1]. The average adult is thought to consume up to several hundred micrograms of phylloquinone per day [1,2]. The approximate vitamin K content of various foods is given in Table 10.4.

Table 10.4 shows that dietary vitamin K is provided mainly by plant foods, especially leafy green vegetables, and certain legumes [3-5]. The richest vegetable sources and the main dietary sources of vitamin K include leafy green vegetables, especially collards, spinach, turnip greens, some salad greens, and broccoli. Oils and margarine represent the second major source of

Table 10.4 Vitamin K Content of Selected Foods

Phylloquinone µg/100 g			
<10	10–50	>100	>200
Milk	Asparagus	Cabbage	Broccoli
Butter	Celery	Lettuce	Kale
Eggs	Green beans	Brussels sprouts	Swiss chard
Cheese	Avocado		Turnip
Meats	Kiwi	Mustard greens	Watercress
Fish	Pumpkin (canned)		greens
Corn			Collards
Cauliflower	Peas		Spinach
Grains	Peanut butter		Salad greens
Fruits (most)	Lentils		
Tea (brewed)	Kidney beans		
	Pinto beans		
	Soybeans		
	Coffee (brewed)		

Source: Adapted from Booth et al. Vitamin K1 (phylloquinone) content of foods. J Food Comp and Anal 1993;6:109–20.

the vitamin [1]. Rapeseed and soybean oils are particularly rich (142–200 µg/100 g) in phylloquinone [4]. Olive oil contains 55 µg phylloquinone/100 g oil. Sunflower, safflower, walnut, and sesame oils provide only 6 to 15 µg phylloquinone/100 g, and peanut and corn oils contain <3 µg/100 g [4]. Smaller amounts of phylloquinone are found in cereals, fruits, dairy products, and meats. Exposure of the vitamin to light and heat can result in significant vitamin K destruction [4].

Menaquinones are synthesized by a variety of facultative and obligate anaerobic bacteria that reside in the lower digestive tract, although small amounts of menaquinones also may be found in a few foods such as liver, fermented cheeses, and soybean products [6]. Examples of menaquinone producing obligate anaerobes include *Bacteroides*, *Bacillus fragilis*, *Eubacterium*, *Propionibacterium*, and *Arachnia*. *Escherichia coli*, a facultative anaerobe, also produces menaquinone [7]. Bacterial synthesis of vitamin K is not sufficient to meet the needs of healthy children or adults [1].

Although rarely needed, vitamin K supplements as phylloquinone (such as Mephyton and Konakion) are available. Water-soluble forms of the vitamin (such as AquaMephyton, Synkayvite, and Kappadione) are also manufactured for people with fat malabsorptive disorders.

ABSORPTION, TRANSPORT, AND STORAGE

Phylloquinone is absorbed from the small intestine, particularly from the jejunum. Absorption of vitamin K occurs as part of micelles and thus is enhanced by the presence of dietary fats, bile salts, and pancreatic juice.

Menaquinones that are synthesized by some bacteria in the lower digestive tract are absorbed by passive diffusion from the ileum and colon; however, the ability to absorb

and use the bacterially produced vitamin varies considerably from human to human and has been difficult to determine accurately [1].

Within the intestinal cell, phylloquinones are incorporated into the chylomicron that enters the lymphatic and then the circulatory system for transport to tissues. Chylomicrons transport most phylloquinone [8]. Chylomicron remnants deliver vitamin K to the liver. Absorbed menadiones are alkylated in the liver and then, along with phylloquinone and menaquinone, incorporated into very low density lipoproteins, and ultimately carried to extrahepatic tissues in low-density and high-density lipoproteins [8].

Vitamin K is stored in several tissues. Phylloquinones are found in higher concentrations in the liver, with lesser amounts in the heart, lungs, kidneys, among other tissues [9,10]. Hepatic concentrations of phylloquinone range from about 2 to 20 ng per g liver and are about 10 times lower than those of the menaquinones [6,11]. Menaquinone-4 (MK-4) synthesis from menadione has been demonstrated with MK-4 found in a variety of tissues, including the pancreas, salivary glands, brain, and bone [9,10]. Circulating plasma concentrations of phylloquinones range from about 0.15 to 1.15 ng/mL [12]. The body pool size of vitamin K, estimated at 50 to 100 µg, is quite low for a fat-soluble vitamin and smaller than that of vitamin B₁₂ [13]. Turnover of vitamin K is rapid, approximately once every 2.5 hours [13].

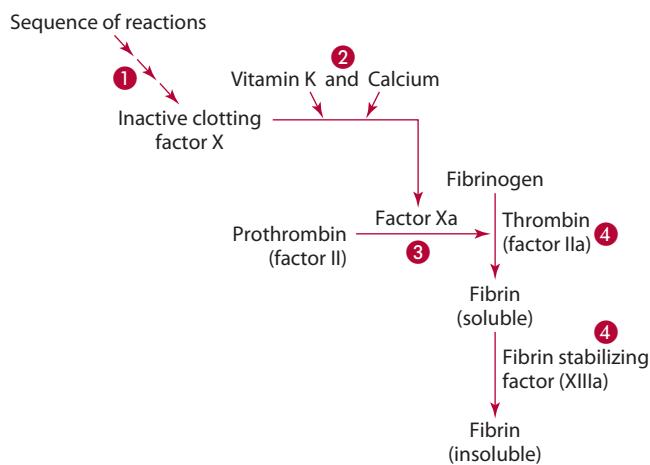
FUNCTIONS AND MECHANISMS OF ACTION

Vitamin K is necessary for the posttranslational carboxylation of specific glutamic acid (glutamyl) residues in proteins to form γ -carboxyglutamate residues, which enable the protein to bind to calcium and interact with other compounds. These interactions are necessary for blood clotting (hemostasis) and bone mineralization, among other processes including apoptosis, arterial calcification, signal transduction, and growth control. The role of vitamin K in blood clotting and vitamin K's possible roles in bone and nonosseous tissues are reviewed here.

Vitamin K and Blood Clotting

The vitamin K-dependent posttranslational carboxylation of glutamyl residues forms γ -carboxyglutamate on four major proteins required for the coagulation of blood. The four vitamin K-dependent blood-clotting proteins, called *factors*, are factors II (prothrombin), VII, IX, and X. In addition, proteins C, S, and Z, also require vitamin K for carboxylation, but these proteins function to inhibit the coagulation process (i.e., are anticoagulants).

Overview of Blood Clotting For blood to clot, fibrinogen, a soluble protein, must be converted to fibrin, an insoluble fiber network, as shown in Figure 10.21. Thrombin catalyzes the proteolysis of fibrinogen to yield fibrin. Fibrin



- ① A series of reactions generates the inactive clotting factor X.
- ② Vitamin K and calcium activate inactive clotting factors to make them active (designated by a lower case a).
- ③ Active clotting factor Xa converts prothrombin to thrombin.
- ④ Thrombin and fibrin stabilizing factor XIIIa form fibrin which aggregates to produce an insoluble clot and stops bleeding.

Figure 10.21 An overview with an emphasis on the final steps in blood clotting.

molecules aggregate to form a polymer, which then undergoes cross-linking by fibrin stabilizing factor (activated by thrombin or factor XIII) to form an insoluble clot and stop bleeding (hemorrhage).

Thrombin, however, circulates in the blood as prothrombin, an inactive enzyme (zymogen). Two pathways, extrinsic and intrinsic, can be used to generate prothrombin and thus thrombin for blood clotting. The reactions needed to produce thrombin occur in a cascade as shown in Figures 10.21 and 10.22 and described below. In the intrinsic pathway (as shown in step 1 Figure 10.22), the coagulation process is initiated by adsorption of factor XII or XI (both of which circulate in the blood) onto a substance such as collagen. Upon contact, factor XII or XI becomes activated, as indicated by the letter *a* next to the factor. The cascade proceeds in a stepwise process as shown in Figure 10.22:

- ② X_{1a}, now an active protease, associates with a protein cofactor and its substrate, factor IX, a vitamin K-dependent carboxylated protein in contact with calcium. Factor IX is converted to IX_a by factor X_{1a}.
- ③ – ④ Factor IX_a continues the cascade of reactions by associating with factor VIII_a and its substrate, factor X. Factor VIII_a is synthesized from factor VIII by thrombin (II_a). Factor X is converted to X_a by active protease factor IX_a.
- ⑤ Factor X_a is another vitamin K-dependent carboxylated protein that interacts with calcium and phospholipids. It also associates with factor Va and its substrate prothrombin (factor II), another vitamin K-dependent carboxylated protein. Factor Va is generated from factor

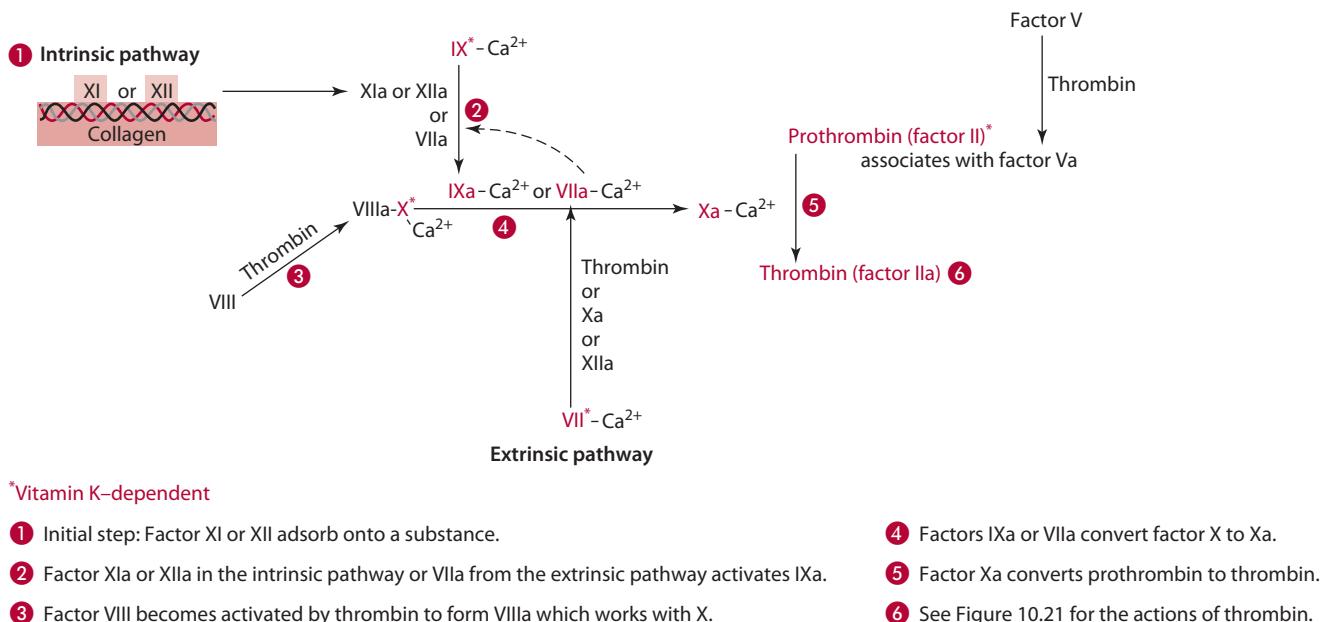


Figure 10.22 The roles of vitamin K-dependent clotting factors in the coagulation of blood.

V by thrombin (IIa). Factor Xa hydrolyzes prothrombin (factor II) to produce thrombin (IIa).

- ⑥ Thrombin catalyzes the conversion of fibrinogen to fibrin for clot formation.

Other proteins, designated C, S, and Z, also have been identified as vitamin K-dependent carboxylated proteins. The function of protein Z is unknown, but proteins C and S appear to inhibit the blood-clotting process and thus exhibit anticoagulant functions. Protein C associates with thrombomodulin and calcium, and in the presence of thrombin it can be converted to protein Ca. This active protease, in association with calcium and protein S, inactivates factors VIIa and Va to disrupt the clotting process. Protein S has been found not only in the blood but also in bone and thus may have other functions.

In the extrinsic pathway, blood-clotting proteins interact with tissue factors in a second cascade of reactions that intercepts with the intrinsic pathway as shown in Figure 10.22 and briefly described here. In the extrinsic pathway, factor VII is converted to VIIa by a variety of blood-clotting proteases, including thrombin and factors Xa and XIIa. Factor VIIa, a carboxylated vitamin K-dependent protein, in association with calcium and a tissue factor, can convert factor IX (a vitamin K-dependent protein associated with calcium) to IXa and can convert factor X (vitamin K-dependent protein associated with calcium and phospholipids) to Xa. As in the intrinsic pathway, factor Xa synthesizes thrombin from prothrombin, and thrombin converts fibrinogen to fibrin for clot formation.

The Role of Vitamin K in Carboxylation of Glutamic Acid Residues

This section uses prothrombin as a model to describe the carboxylation process. Remember that in addition to prothrombin (factor II), blood-clotting factors VII, IX, and X, as well as other proteins such as C, S, and Z and those discussed under bone and nonosseous roles of vitamin K, depend on vitamin K for carboxylation. Proteins like prothrombin require a vitamin K-dependent enzyme for the carboxylation of 10 to 12 glutamic acid residues residing in its N-terminal. Once carboxylated, this glutamic acid portion forms γ -carboxyglutamic acid (Gla) (also called γ -carboxy glutamate), as shown in Figure 10.23. The carboxylation is required for the protein to become functional. The enzyme responsible for the γ -carboxylation, called vitamin K-dependent γ -glutamyl carboxylase, is associated with the rough endoplasmic reticulum (where vitamin K-dependent proteins are carboxylated), primarily in the liver. The liver is also where the hemostatic factors are synthesized. The enzyme, however, is found in all human tissues. This widespread occurrence of γ -glutamyl carboxylase suggests that the need for carboxylated proteins that can bind calcium is quite broad.

Gla residues are synthesized posttranslationally as the protein is being secreted out of the cell. All glutamic acid residues must be carboxylated for the protein to function. Gla residues on the protein function to bind calcium. The calcium then mediates the binding of Gla proteins to negatively charged phospholipids on membrane surfaces. This adsorption of specific proteins on phospholipid surfaces is essential in hemostasis, including the initiation, progression, and regulation

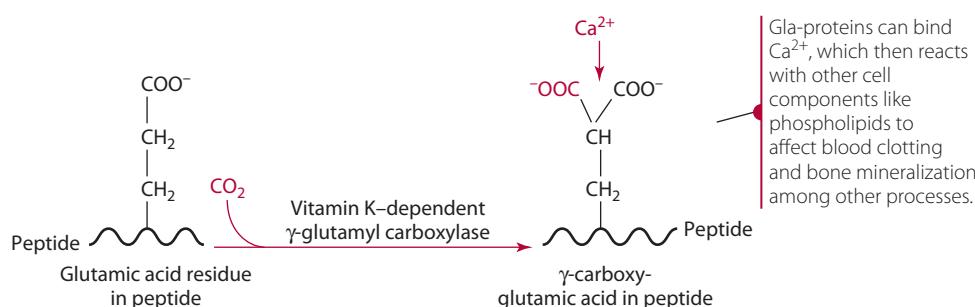


Figure 10.23 Production of γ -carboxyglutamic acid (Gla) via vitamin K-dependent carboxylation.

of blood clotting. In addition, Gla residues bind calcium to hydroxyapatite in the extracellular matrix of bone.

The participation of vitamin K in the carboxylation of proteins is a cyclic process (Figure 10.24) often called the vitamin K cycle. The γ -glutamyl carboxylase enzyme requires dihydrovitamin KH_2 , also known as reduced, dihydroxy, or hydroquinone vitamin K. Thus, for carboxylation to occur, vitamin K is needed in the reduced form, vitamin KH_2 . However, vitamin K is generally present in the body in its oxidized quinone form because of the presence of oxygen in the blood. The steps of the vitamin K cycle are reviewed here and in Figure 10.24.

- Reduction of vitamin K quinone to the active KH_2 form can be accomplished by quinone reductases that require either dithiol (RSH-HSR) or NAD(P)H. The dithiol-dependent quinone reductase appears to be the main physiological pathway for generating vitamin KH_2 from the quinone. (See steps 1 and 2 Figure 10.24.)
- Once KH_2 is present, along with oxygen and carbon dioxide as the carboxyl precursor, γ -glutamyl carboxylase can carboxylate (add a CO_2 onto) the glutamic acid residues on the protein. (See steps 3 and 4 Figure 10.24.)
- The carboxylation of glutamic acid is believed to be coupled with the formation of vitamin K 2,3-epoxide, as illustrated in Figure 10.24 step 5. No adenosine triphosphate (ATP) is required for the carboxylation reaction; the reaction is probably accomplished by the free energy produced through the oxidation of vitamin KH_2 to vitamin K 2,3-epoxide, whereby vitamin K provides reducing equivalents [14].
- As the cycle continues (Step 6 Figure 10.24), vitamin K 2,3-epoxide is converted to vitamin K quinone by an epoxide reductase.
- The quinone is then converted back (See step 1 Figure 10.24) to the dihydroxy (hydroquinone) vitamin K (KH_2) by one of the two quinone reductases, requiring either NAD(P)H or 2 RSH as previously described.

Anticoagulants. Coumadin (warfarin) is an anticoagulant that may be prescribed to people at risk for a thrombotic

event (such as a heart attack). The anticoagulant antagonizes the synthesis of vitamin K. Warfarin, for example, interferes with the dithiol-dependent quinone reductase necessary for reducing oxidized vitamin K to the KH_2 form. Warfarin also may act on the dithiol-dependent epoxide reductase, again preventing KH_2 regeneration [13]. Use of the drug results in undercarboxylation of glutamic acid residues. Ingestion of diets high in vitamin K, as obtained from about a pound of broccoli daily, can lead to warfarin resistance [15]. Thus, people who are taking anticoagulant medications are instructed not to consume large quantities of foods rich in vitamin K.

Vitamin K and Bone and Nonosseous Tissue Proteins

Two vitamin K-dependent proteins have been identified in bone, cartilage, and dentine: osteocalcin (also called bone Gla protein and abbreviated BGP) and matrix Gla protein (MGP). The synthesis of both osteocalcin and MGP appears to be stimulated by active vitamin D, $1,25-(OH)_2D_3$, and by retinoic acid. Osteocalcin is secreted by osteoblasts during bone matrix formation, about at the onset of hydroxyapatite deposition. Osteocalcin comprises about 15% to 20% of noncollagen protein in bone. With vitamin K-dependent carboxylation, the three Gla residues on osteocalcin facilitate the binding of calcium ions in the hydroxyapatite lattice [16]. Although its physiological role remains unclear, osteocalcin appears to be involved in bone remodeling or calcium mobilization.

MGP is found in bone, dentine, and cartilage and is associated with the organic matrix and mobilization of bone calcium. As with osteocalcin, the physiological role of MGP is uncertain. However, messenger RNA for MGP has been found in a variety of tissues, including brain, heart, kidney, liver, lung, and spleen, suggesting a broad role for the protein. Lack of MGP in knockout mice has been associated with extensive arterial calcification [17]. Undercarboxylation of vascular MGP, as would occur with inadequate vitamin K, thus increases calcification of atherosclerotic lesions [7]. Thus, MGP may function to prevent calcium precipitation.

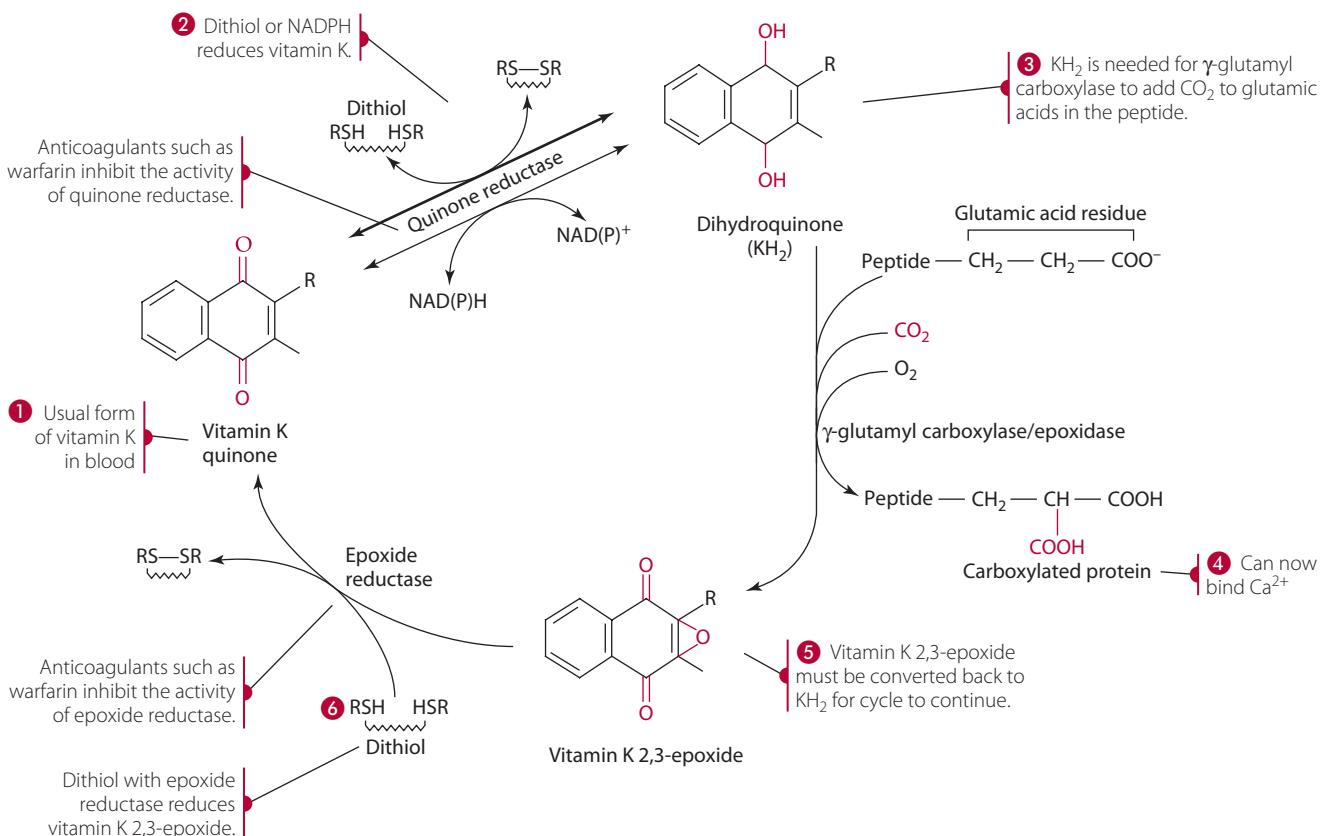


Figure 10.24 The vitamin K cycle.

A third vitamin K-dependent protein, kidney Gla protein (KGP), has been identified in the cortex of the kidney. Further research is needed to delineate the roles of not only KGP but also MGP and osteocalcin.

A role for vitamin K also has been suggested in sphingolipid metabolism. Decreased concentrations of sulfatides and galactocerebroside sulfotransferase in the brain have been documented in those receiving the vitamin K antagonist warfarin.

INTERACTIONS WITH OTHER NUTRIENTS

The fat-soluble vitamins A and E are known to antagonize vitamin K. Excess vitamin A appears to interfere with vitamin K absorption. Vitamin E's antagonistic effects on vitamin K include possible inhibition of vitamin K absorption, function, and metabolism [2,13,18]. Vitamin E or its quinone (α -tocopheryl quinone), for example, is thought to block regeneration of the reduced form of vitamin K or to affect prothrombin formation by another manner [19,20].

A possible interrelationship among vitamins K, D, and A is suggested by their relationship to the mineral calcium. Vitamin D's functions affect calcium metabolism, and vitamin K-dependent proteins bind calcium. Two sites of action of $1,25-(\text{OH})_2 \text{D}_3$ are bone and kidney, and in both these tissues vitamin K-dependent calcium-binding proteins have been identified. Retinoic A as well as $1,25-$

$(\text{OH})_2 \text{D}_3$ has been shown to regulate, in part, production of BGP, MGP, and KGP. Further research is needed to better characterize the interrelationships.

METABOLISM AND EXCRETION

Phylloquinone, degraded much more slowly than menaquinone, is almost completely metabolized to a variety of metabolites (many uncharacterized) before being excreted. The metabolism usually involves oxidation of the phytol side chain at position 3 with subsequent conjugation. Most of the metabolites of phylloquinone are conjugated with glucuronides for excretion primarily in the feces by way of the bile, with some metabolite excretion in the urine.

Relatively little is known about the metabolism and excretion of menaquinone. Studies suggest that menadione is rapidly metabolized to menadiol, which then reacts with phosphate, sulfate, or glucuronide. Menadiol phosphate and menadiol sulfate are excreted in the bile (and thus ultimately in the feces) and in the urine; menadiol glucuronides are excreted mostly in the feces by way of the bile.

ADEQUATE INTAKE

Lack of data has hampered the Food and Nutrition Board in its efforts to estimate requirements for vitamin K [1]. Adequate intake (AI) recommendations of 120 and 90 μg

for adult males and females (including those who are pregnant or lactating), respectively, have been set based on the highest dietary intakes of vitamin K by healthy people obtained from the National Health and Nutrition Examination Survey (NHANES) III [1]. Values of vitamin K intake were rounded up to the nearest 5 µg for setting the AIs [1]. Recommended AIs for children age 1 to 3 and 4 to 8 years are 30 µg and 55 µg, respectively, and those for children age 9 to 13 and 14 to 18 years are 60 µg and 75 µg, respectively [1]. Recommendations for children also are based on the highest median intake for each age group found in the NHANES III [1].

The 2001 recommendations for vitamin K intake are higher than those established for the first time by the board in 1989. The 1989 recommended dietary allowances for vitamin K for adult males and females were 80 µg and 65 µg per day, respectively [21]. In the past, a range of intakes has been suggested based on the assumption that a substantial amount of vitamin K could be supplied by intestinal bacteria. However, it is now recognized that bacterial-generated menaquinones are generally not sufficiently produced or used to maintain adequate vitamin K status [1]. In addition, metabolic studies suggest that the current recommendations for vitamin K are not adequate to maximize carboxylation of proteins needed for bone health [2,22,23].

DEFICIENCY

A deficiency of vitamin K is unlikely in healthy adults. The population groups that appear to be most at risk for a vitamin K deficiency are newborn infants and people with severe gastrointestinal malabsorptive disorders or people being treated chronically with antibiotics. Newborns are particularly at risk because their food is limited to milk, which is low in vitamin K; their stores of the vitamin are low because inadequate amounts cross the placenta; and their intestinal tract is not yet populated by vitamin K-synthesizing bacteria. Supplementation with vitamin K is considered advisable for all newborns; currently, intramuscular injection of 0.5 to 1 mg phylloquinone shortly after birth is recommended for all infants [1].

People consuming vitamin K-poor diets and on prolonged sulfa and antibiotic drug therapy are at risk for vitamin K deficiency owing to the coupled effects of low dietary intake and antibiotic-induced destruction of gastrointestinal bacteria that manufacture the vitamin and contribute a source of vitamin K. Other conditions and populations associated with increased need for vitamin K intake include those with fat malabsorptive disorders such as biliary fistula, obstructive jaundice, steatorrhea or chronic diarrhea, intestinal bypass surgery, chronic pancreatitis, and liver disease. Because vitamin K is fat soluble, it is absorbed best with dietary fat. Consequently, people who malabsorb fat also malabsorb fat-soluble vitamins.

Subclinical vitamin K deficiency has been induced in healthy adults fed a diet providing only about 10 µg phylloquinone per day [24]. The 13-day low-vitamin K diet resulted in a significant reduction in plasma vitamin K concentrations. Urinary γ -carboxyglutamate excretion significantly decreased in younger subjects but remained unchanged in older adults. Prothrombin time did not change; however, descarboxyprothrombin (undercarboxylated prothrombin) concentrations increased significantly in subjects [24]. Severe vitamin K deficiency is associated with bleeding episodes (hemorrhage) caused by prolonged prothrombin time. The undercarboxylated blood-clotting factors cannot effectively bind calcium and interact with cell membrane phospholipids exposed on tissue injury, an interaction necessary for thrombin generation and clot formation.

A relationship between vitamin K and osteoporosis has been suggested. Subclinical vitamin K deficiency may be associated with alterations in bone mineral density and increased fracture rates, although the results of studies are not consistent.

TOXICITY

Ingestion of large amounts of phylloquinone and menaquinone has caused no symptoms of toxicity, and no tolerable upper intake level for vitamin K has been established by the Food and Nutrition Board [1,25]. The synthetic product menadione, however, can cause liver damage when ingested in relatively large amounts. Toxic effects reported in infants supplemented with menadione include hemolytic anemia, hyperbilirubinemia, and severe jaundice [20,25]. Menadione is thought to combine with sulphydryl groups such as those in glutathione, resulting in glutathione oxidation and excretion and ultimately membrane damage induced by phospholipid oxidation.

ASSESSMENT OF NUTRITION

Plasma or serum concentrations of phylloquinone have been shown to reflect recent (within about 24 hours) intake of the vitamin [26]. Whole blood clotting times and prothrombin (or other blood-clotting proteins) time are routinely measured and used to identify potential deficiency of vitamin K. Prothrombin time measures the time required for a fibrin clot to form following the addition of calcium and other substances to citrated plasma. A normal prothrombin time is considered to be between about 11 and 14 seconds; times greater than 25 seconds are associated with major bleeding and may indicate possible vitamin K deficiency. This test is considered relatively insensitive, however, because plasma prothrombin concentrations typically must decrease considerably (sometimes 50% or more) prior to any effects on prothrombin time [27].

Another means of assessing vitamin K status is to measure undercarboxylated vitamin K-dependent proteins, such as prothrombin and osteocalcin, or the ratio of under- to fully carboxylated proteins [26]. Vitamin K deficiency results in the secretion of under- or partially carboxylated proteins into the blood. These undercarboxylated proteins, which have been called protein-induced vitamin K absence or antagonism (PIVKA), have been shown to respond to dietary changes in vitamin K [1].

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The Antioxidant Nutrients, Reactive Species, and Disease

Although different sections in several chapters of this book have addressed the roles of selected nutrients as they relate to antioxidant function, nowhere is this information brought together to provide a comprehensive review of how these individual nutrients function together to protect the body from destructive radicals and destructive nonradical species. That is the purpose of this Perspective, which first reviews free radical chemistry. It next addresses how free radicals and selected nonradicals are generated in the body, the damage caused by reactive oxygen and nitrogen species, and finally how the antioxidant nutrients function together to eliminate destructive radical and nonradical species.

Free Radical Chemistry

Back in probably one of your first chemistry courses, you learned about atoms. It is here that a brief review of free radical chemistry begins. Atoms contain protons and neutrons, which are found in the nucleus. You may remember that the atomic weight of an element is a function of its number of protons and neutrons, whereas the atomic number represents solely the number of protons. Atoms also have electrons, which revolve in orbitals (also called shells) around the nucleus. An atomic orbital holds a maximum of two electrons. These electrons are generally found in pairs in the orbitals. The term *free radical* represents an atom or molecule that has one or more unpaired electrons. The unpaired electron is found alone in the outer orbital and is usually denoted by a superscript dot next to the element. The **superoxide radical** is denoted with a superscript dot (O_2^-), or a superscript dash (O_2^-), or both (O_2^{\bullet}). The imbalance in electrons in the orbitals results in most cases in the high reactivity of the free radicals. Free radicals that contain oxygen are called reactive oxygen species (ROS), and free radicals containing nitrogen are called reactive nitrogen species (RNS). The term *reactive* is most appropriately used when comparing different radicals, because reactivity with other compounds is relative. The terms *reactive oxygen species* and *reactive nitrogen species*, however, include not only free radicals containing oxygen and nitrogen, respectively, but also nonradicals, as shown in Table 1.

The radicals listed in Table 1 do not include all free radical or reactive species. Oxygen itself is a biradical because it has two unpaired electrons, residing in separate orbitals, that cannot form a pair. An example of a reactive sulfur species radical is thiyl (RS^\bullet), generated from amino acids and thiols. Trichloromethyl (CCl_3^\bullet),

Table 1 Some Reactive Oxygen and Nitrogen Species

Reactive Oxygen Species		Reactive Nitrogen Species	
Oxygen-Containing Radicals	Oxygen-Containing Nonradicals	Nitrogen-Containing Radicals	Nitrogen-Containing Nonradicals
Superoxide O_2^-	Ozone O_3	Nitric oxide NO^\bullet	Nitrous acid HNO_2
Hydroxyl $^{\bullet}OH$	Singlet oxygen 1O_2	Nitrogen dioxide NO_2^\bullet	Peroxynitrite $ONOO^-$
Hydroperoxyl HO_2^\bullet	Hypochlorous acid $HOCl$	Alkyl peroxynitrite $LOONO_2^\bullet$	
Alkoxy LO^\bullet or RO^\bullet	Hydrogen peroxide H_2O_2		
Peroxy LO_2^\bullet or RO_2^\bullet			

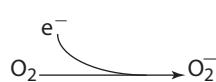
formed during metabolism of carbon tetrachloride (CCl_4) by cytochrome P_{450} enzymes in the liver, is a chloride-based carbon-centered radical, meaning that the unpaired electron resides on the carbon atom.

Generation of Reactive Species

A variety of different reactive species are generated daily from multiple sites in the body. In general, the reactive oxygen species are formed on exposure to substances such as smog, ozone, chemicals, drugs, radiation, and high levels of oxygen, among others, and during normal physiological processes, especially in the defense against microbes and other foreign substances. Radicals also breed more radicals, as seen in several of the reactions shown in this Perspective. Production of the superoxide radical; hydrogen peroxide; the hydroxyl radical; the peroxy, hydroperoxy, and carbon-centered (alkyl) radicals; and lipid peroxides is reviewed in this section and shown in Figure 1. A few reactive nitrogen species radicals also are discussed.

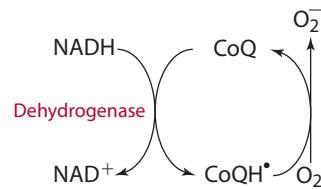
The Superoxide Radical

The superoxide radical (designated hereafter as O_2^-) is an oxygen-centered radical; that is, the unpaired electron resides on the oxygen. Remember that molecular oxygen has two unpaired electrons in different orbitals. The addition of an electron to molecular oxygen leaves only one unpaired electron.



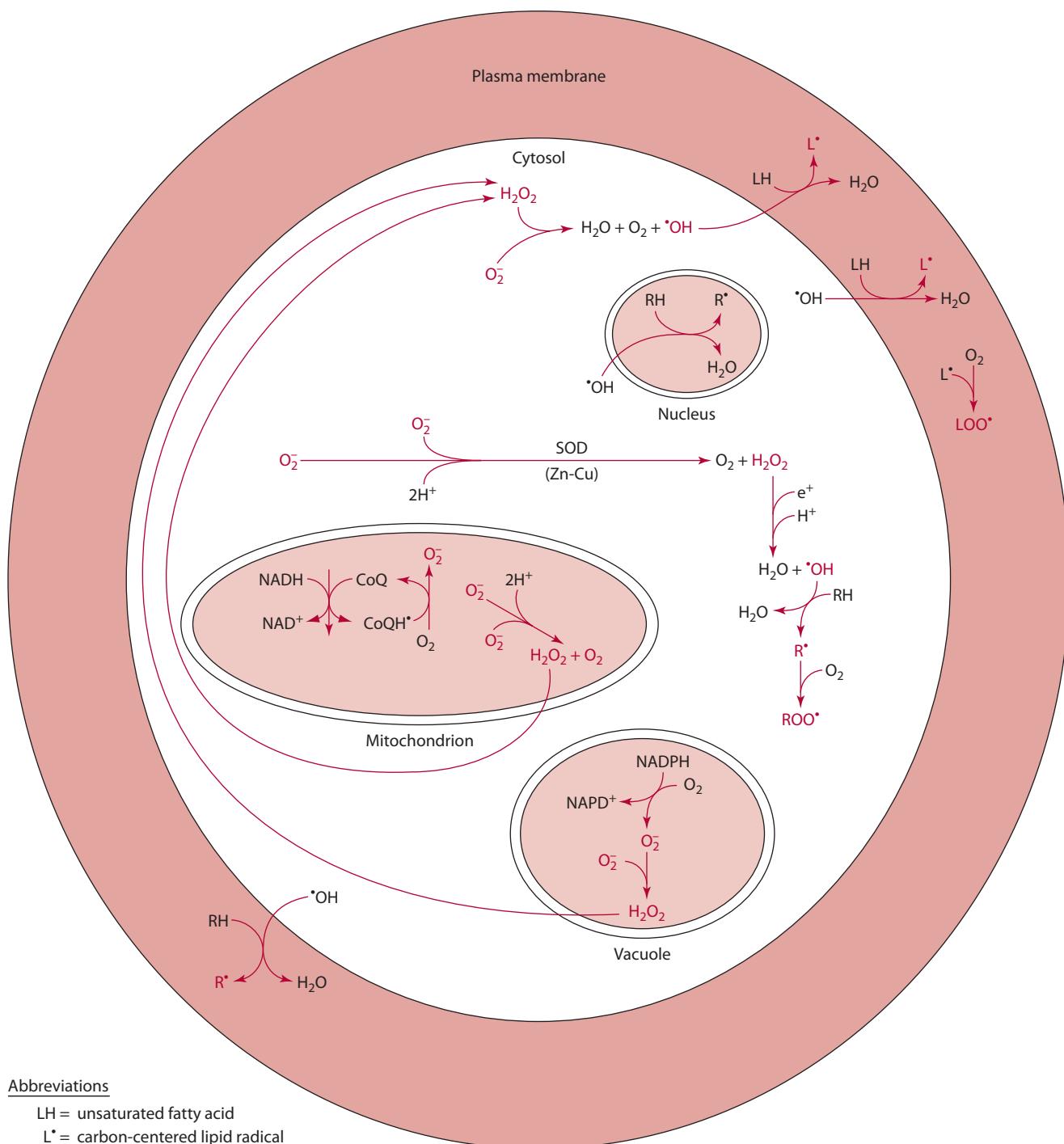
Superoxide radicals can be made when oxygen molecules (O_2) react with different compounds, such as with the catecholamines epinephrine and dopamine or with the vitamin folate, as tetrahydrofolate. The electron

transport chain also accidentally produces superoxide radicals as a result of autoxidation reactions and leaking of electrons from the electron transport chain onto oxygen—that is, a one-electron reduction of oxygen to generate the superoxide radical. This leaking of electrons onto oxygen occurs during the passage of electrons from $CoQH^\bullet$ (coenzyme Q) as part of the electron transport chain (see Chapter 3). In the electron transport chain, electrons ultimately are transferred to oxygen (O_2) for ATP production; however, upon interaction between $CoQH^\bullet$ and O_2 , shown here, the superoxide radical is formed:



Cytochrome P_{450} enzymes also can generate superoxide radicals. These heme enzymes found in the endoplasmic reticulum membrane consist of a cytochrome P_{450} reductase that transfers electrons from $NADPH$, and a second cytochrome P_{450} that binds molecular oxygen and the substrate being hydroxylated. A variety of substrates, including fatty acids, steroids, and therapeutic drugs, are hydroxylated by this system. The reactions catalyzed by some of the cytochrome P_{450} enzymes convert nonpolar compounds to polar compounds. This change in polarity enables elimination (fecal or urinary) of the compound from the body.

Superoxide radicals also are produced in substantial quantities in activated white blood cells, such as macrophages, monocytes, and neutrophils conducting phagocytosis, to assist in destroying foreign substances such as bacteria and viruses. The superoxide radicals in

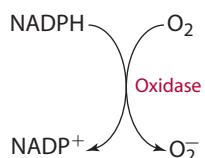


Abbreviations

- LH = unsaturated fatty acid
- L^\bullet = carbon-centered lipid radical
- RH = organic compound (e.g., amino acid, nucleic acid)
- O_2^- = superoxide radical
- $\cdot\text{OH}$ = hydroxy radical
- R^\bullet = carbon-centered nonlipid radical
- H_2O_2 = hydrogen peroxide
- ROO^\bullet = nonlipid peroxy radical
- LOO^\bullet = peroxy radical

Figure 1 Generation of reactive species.

these cells are needed for the subsequent production of other toxic reactive oxygen species, such as hydrogen peroxide (H_2O_2), to further help destroy foreign bacteria and other organisms. In addition, superoxide radicals generated by neutrophils heighten the inflammatory response by acting as a chemoattractant for other neutrophils. Production of superoxide radicals in activated white blood cells is thought to begin with the action of NADPH oxidase while a foreign substance is being engulfed by a white blood cell. Specifically, the NADPH oxidase reduces oxygen and produces tremendous quantities of superoxide radicals. This reaction is shown here:

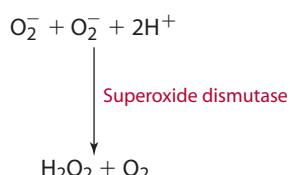


The radicals in turn help kill the bacteria and other foreign substances. The extensive oxygen-requiring process by which white blood cells destroy organisms is sometimes called the *respiratory burst*.

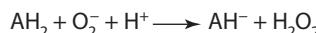
Although superoxide radicals help to destroy bacteria, viruses, fungi, and the like, in white blood cells, these same radicals can do harm. They are a potent initiator of chain reactions and can lead to the production of other reactive oxygen species, such as hydrogen peroxide and the hydroperoxyl radical. The superoxide radical also can react with nitric oxide ($\bullet NO$) to generate several reactive nitrogen species, including peroxynitrite. Fortunately, superoxides are not lipid-soluble and thus do not diffuse too far away from their site of production.

Hydrogen Peroxide

Hydrogen peroxide is not a radical, because it has no unpaired electrons, but it is considered a reactive oxygen species and easily diffuses throughout cells, causing damage. Hydrogen peroxide is generated through the action of the enzyme superoxide dismutase (SOD). This enzyme, which removes superoxide radicals, is found extracellularly and intracellularly, in both the cell cytoplasm and mitochondria. Extracellular and cytosolic SOD require both zinc and copper, whereas mitochondrial SOD is manganese dependent.



Ascorbate (AH_2), like SOD, also can generate hydrogen peroxide while trying to eliminate superoxide radicals. The reaction is as follows:

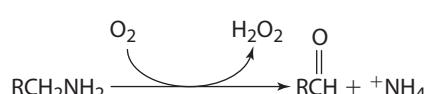


Hydrogen peroxide also is produced in large quantities during the oxidation of compounds in peroxisomes. Peroxisomes are cytoplasmic organelles responsible for the degradation of molecules such as very long chain (20+ carbons) fatty acids, among others.

Other reactions in the body also generate hydrogen peroxide. In trauma or injury such as intestinal or cardiac ischemia (inadequate blood flow and, thus, oxygen supply), for example, many reactive oxygen species are generated, especially hydrogen peroxide. There are three possible reasons for the free radical and nonradical production observed in ischemic tissue. First, activation of neutrophils by compounds released by the damaged tissues results in the generation of hydrogen peroxide and superoxide radicals [1,2]. Second, injury may result in disruption of the respiratory chain, with more electrons leaked to oxygen for superoxide radical formation [1–3]. Third, for tissues such as the intestine and possibly the endothelial cells of blood vessels, the generation of xanthine oxidase results in free radical formation [1–3]. During ischemia, xanthine dehydrogenase is converted into xanthine oxidase by oxidation of sulfhydryl groups, proteolysis, or both. Both xanthine dehydrogenase and xanthine oxidase require molybdenum and riboflavin as FAD and catalyze hypoxanthine and xanthine degradation. In hypoxic tissue, ADP is degraded (because of lack of oxygen for ATP generation), producing lots of hypoxanthine.

During medical treatment of ischemia, oxygen is administered to the patient. Although the oxygen helps to prevent organ damage, the large quantities of oxygen given with reperfusion provide xanthine oxidase with the oxygen (O_2) needed to oxidize hypoxanthine and xanthine and also generate H_2O_2 , as shown in Figure 12.23. The production of these reactive oxygen species by xanthine oxidase can further damage the already injured tissue [1,2].

Other cellular oxidases, such as amine oxidase (which is copper dependent), also generate hydrogen peroxide. The reaction catalyzed by amine oxidase, found in the blood and body tissues, is as follows:



Concentrations of hydrogen peroxide, like superoxide radicals, need to be controlled in the body cells to prevent cellular destruction. Hydrogen peroxide easily diffuses in water and in lipids, within cells and to tissues, to cause damage. It also can react with superoxide radicals to produce a highly reactive and destructive hydroxyl radical.

The Hydroxyl Radical

The hydroxyl radical ($\bullet OH$) is an oxygen-centered radical. It can be produced when the body is exposed to γ rays, low wavelength electromagnetic radiation. These rays split water in the body to form the hydroxyl radical:



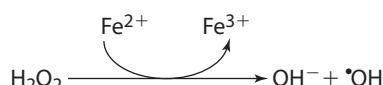
Hydroxyl radicals also are produced from a reaction between hydrogen peroxide and superoxide radicals (known as the Haber Weiss reaction), as shown here,



or from other electrons and protons, as shown here:



Hydrogen peroxide in contact with free ferrous iron can result in the formation of hydroxyl radicals. However, iron normally is bound to proteins and is not found free in cells. If the iron is freed (protein bound— $Fe^{3+} + O_2^- \rightarrow$ free $Fe^{3+} + O_2^- \rightarrow O_2 + Fe^{2+}$), the following reaction, known as the Fenton reaction, may occur and generate the hydroxyl radical:



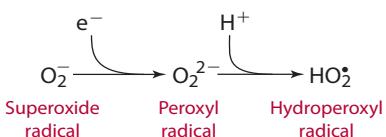
In this reaction, the hydrogen peroxide is functioning as an iron-oxidizing agent. Free copper also is able to react with hydrogen peroxide, but copper, like iron, is bound to proteins *in vivo*.

The hydroxyl radical, thought to be one of the most potent or reactive radicals, rapidly attacks (by taking electrons) virtually all molecules in the body [4]. In fact, the hydroxyl radical is thought to be a

major initiator of lipid peroxidation. It also reacts with nucleic acids in DNA, forming 8-hydroxyguanosine (a compound used to estimate DNA damage). Hydroxyl radicals fragment proteins, primarily at proline and histidine residues, triggering damage and premature degradation of the protein. Thus, removing free hydroxyl radicals is important to prevent destruction of cell components.

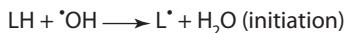
Peroxyl, Hydroperoxyl, and Carbon-Centered Radicals and Lipid Peroxides

Peroxyl (O_2^-) and hydroperoxyl (HO_2^{\cdot}) radicals (oxygen centered) can be formed in the body from superoxide radicals reacting with additional electrons and hydrogen, as shown here:

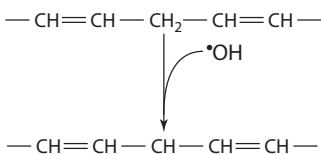


The peroxy radical, as well as the hydroxyl and alkoxyl radicals, is more reactive than the superoxide radical [4].

Lipid carbon-centered radicals (L^{\cdot}) are produced in the body when radicals such as hydroxyl radicals ($\cdot OH$) attack polyunsaturated fatty acids (LH) in the phospholipids of membranes or attack other organic compounds. The initiation reaction in the attack of a polyunsaturated fatty acid may be written as follows:



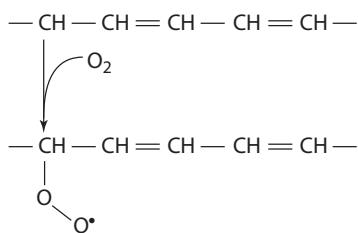
Alternately, the reaction may be viewed showing part of the polyunsaturated fatty acid, as shown here:



Propagation follows the initiation step, with products formed in one reaction being used as reactants in another reaction. Oxygen, for example, can react with the lipid carbon-centered radical to generate a lipid peroxy radical as follows:



or alternately, this reaction may be expressed as follows:



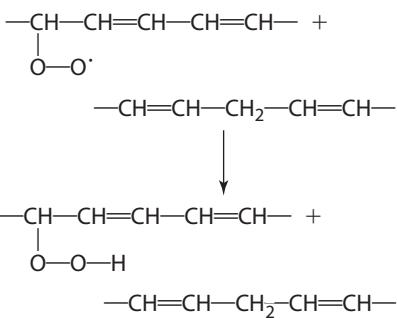
Oxygen also can react with polyunsaturated fatty acids (LH) to form carbon-centered radicals and hydroperoxy radical radicals:



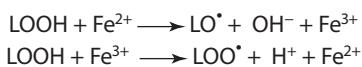
In additional propagation reactions, lipid peroxy radicals (LOO^{\cdot}) may attack (abstract a hydrogen atom or proton from) other polyunsaturated fatty acids (L'H) in cell membranes to generate lipid peroxides (LOOH) and another carbon-centered radical.



This reaction may be depicted as follows:



Should lipid peroxides (LOOH), also known as peroxidized fatty acids, come in contact with free iron, for example, alkoxyl (LO^{\cdot}) and peroxy (LOO^{\cdot}) radicals also can be generated, as shown in these two reactions:



Like peroxy radicals, the alkoxyl radical can in turn initiate chain reactions with other polyunsaturated fatty acids in membranes, as follows:



However, again it is important to note that in vivo, little or no free iron appears to be available to initiate such reactions.

Singlet Molecular Oxygen

Singlet molecular oxygen (1O_2) possesses higher energy and is more reactive than ground-state oxygen. Specifically, in singlet oxygen, the peripheral electron in the oxygen structure is excited to an orbital above the one it normally occupies [4]. This excited form of oxygen can be generated from lipid peroxidation of membranes by enzymatic reactions, such as occur between hydrogen peroxide and hypochlorous acid in the respiratory burst in white blood cells (i.e., $H_2O_2 + HOCl \longrightarrow {}^1O_2 + H_2O + HCl$), or through photochemical reactions, as shown:



Singlet oxygen, being a reactive oxygen species, can, like free radicals, damage cells and tissues unless it is removed from the body.

Nitric Oxide

Nitric oxide ($\cdot NO$) is a widely studied and known vasorelaxant that functions in cells through activation of guanylate cyclase, increasing cyclic GMP concentrations and thus mediating a cascade of cell signals. Nitric oxide's role as a vasorelaxant is applied in medicine. Nitroglycerin, for example, taken by people experiencing ischemic chest pain (angina), generates nitric oxide in the body, which relaxes coronary blood vessels and increases blood (oxygen) flow to the heart. Nitric oxide also is associated, however, with other beneficial and detrimental effects at the vascular and cellular levels. For example, nitric oxide can react with oxygen to form nitrogen dioxide ($\cdot NO + O_2 \longrightarrow \cdot NO_2$), another reactive nitrogen species. Moreover, when nitric oxide reacts with superoxide radicals (O_2^-), peroxy nitrite ($ONOO^-$) is generated; peroxy nitrite acts as an oxidizing agent in the body. Alternately, by removing superoxide radicals and other radicals, nitric oxide can be viewed as an eliminator or terminator of free radicals (as discussed further under elimination of lipid peroxides). Thiols (RSH) also can react with nitric oxide, as shown in the general reaction: $\cdot NO + RSH^- \longrightarrow RSNO + O_2 + H^+$. Nitrosothiols ($RSNO$) can then attack other compounds or terminate by combining with another thiol ($R'SH$) to produce $RSH + R'SNO$ or $RSSR' + HNO$.

Peroxynitrite

Peroxynitrite ($ONOO^-$), formed by reactions between nitric oxide and superoxide radicals, is a strong oxidant. It directly acts on compounds (such as proteins) in the

body, attacking cysteine, methionine, and tyrosine and causing damage. Some peroxy nitrite decomposes, generating more destructive radicals—the hydroxyl radical ($\cdot\text{OH}$) and nitrogen dioxide ($\cdot\text{NO}_2$). Alternately, and, more likely in human tissues and fluids, peroxy nitrite reacts with carbon dioxide (CO_2) to produce carbonate ($\text{CO}_3^{\cdot-}$) + nitrogen dioxide ($\cdot\text{NO}_2$). Both carbonate and nitrogen dioxide preferentially react with nutrients, such as lipids and amino acids within proteins (primarily tyrosine, tryptophan, and cysteine), to form nitrated molecules.

Nitrogen Dioxide and Peroxynitrate

Nitrogen dioxide ($\cdot\text{NO}_2$) is a free radical and a fairly potent oxidant of molecules. It is formed when nitric oxide reacts with oxygen ($\cdot\text{NO} + \text{O}_2 \longrightarrow \cdot\text{NO}_2$). Nitrogen dioxide, for example, in addition to co-acting with carbonate ($\text{CO}_3^{\cdot-}$) radical to produce nitrated compounds, reacts with unsaturated fatty acids by abstracting a hydrogen atom and induces isomerization of *cis*-double bonds in unsaturated fatty acids by a reversible addition reaction. These actions damage the lipids and, if the lipids are part of a cell membrane, damage the membrane. Peroxynitrate ($\text{O}_2\text{NNO}^{\cdot-}$) is made from the reaction between nitrogen dioxide and a superoxide radical. It typically decomposes to form singlet oxygen and $\text{NO}_2^{\cdot-}$.

Damage Due to Reactive Species

Once formed, free radicals attack, taking electrons from cell constituents (including nucleic acid in DNA in the nucleus of cells). They also take electrons from proteins (especially amino acids such as tyrosine, tryptophan, proline, histidine, or arginine and those with sulphydryl groups, such as cysteine) and polyunsaturated fatty acids (PUFAs) in cell membranes or in the membranes of intracellular organelles, such as the nucleus, mitochondria, or endoplasmic reticulum. Hydroxyl radical-induced changes in purine and pyrimidine bases in DNA may lead to mutations or breakages, which if not repaired may result, for example, in cancer [3]. Attack on amino acids in proteins by reactive oxygen species may break the peptide bonds in the protein backbone or disrupt the protein structure. Oxidative damage to proteins may cause cross-linking between amino acids, or aggregation, resulting in changes in the secondary or tertiary structures. Such events may even lead to premature degradation of the protein. Free radical attack on polyunsaturated fatty acids present in the phospholipid portion of the cell membranes can lead to degradation of the lipid. Extensive damage in a

red blood cell, for example, may cause hemolysis of the membrane and thus the cell [5]. Aqueous peroxy and peroxy nitrite radicals may induce oxidation of LDLs. Furthermore, radicals give rise to more radicals and thus, more damage.

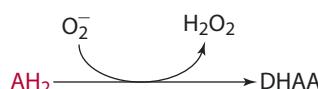
Antioxidant Nutrient Functions

Overproduction of reactive oxygen and nitrogen species and their attack on DNA, proteins, and polyunsaturated fatty acids have been implicated as a cause of or contributor to a variety of conditions and diseases such as cancer, heart disease, cataracts, and complications of diabetes mellitus, among others. Vitamins, along with several other antioxidant compounds, help control or eliminate free radicals. However, the term *antioxidant* is a bit of a misnomer, because once the antioxidant works, the antioxidant itself becomes a radical. Some have suggested that the term *redox agent* be used instead of the term *antioxidant*. Whether or to what extent an overproduction of vitamin radicals may be associated with diseases is unclear; however, the results of many clinical trials providing antioxidant vitamins to treat or prevent various diseases have failed to show beneficial results, and some have even found that supplementation was detrimental to health. The destruction of reactive species by some of the antioxidant nutrients and compounds is reviewed in this section and shown in Figure 2.

Elimination of Superoxide Radicals

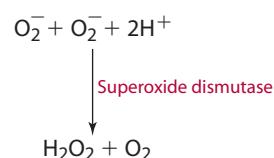
The primary mechanism by which the body gets rid of superoxide radicals is by converting the superoxide radicals to other compounds. Several antioxidant nutrients help dispose of superoxide radicals. These nutrients include vitamin C and three minerals (zinc, copper, and manganese) that function as cofactors for enzymes involved in oxidant defense.

Vitamin C (ascorbate or ascorbic acid), being water soluble and hydrophilic, is found in the aqueous parts of the body, such as the blood or the cytoplasm of the cells. Ascorbate (AH_2) can provide electrons to reduce the superoxide radical and form hydrogen peroxide and dehydroascorbate (DHAA).



The conversion of superoxide radicals to hydrogen peroxide also is accomplished by the action of an enzyme, superoxide dismutase (SOD). In fact,

superoxide dismutase works considerably faster than vitamin C in inactivating superoxide radicals. As previously mentioned, superoxide dismutase is found extracellularly as well as intracellularly. The extracellular form is found in exceptionally high concentrations in arterial blood vessels. Both the extracellular and the cytosolic forms of the enzyme depend on the presence of two minerals, zinc and copper. SOD in the mitochondria depends on manganese for activity. Thus, zinc, copper, and manganese are important minerals in the body's oxidant defense system. Superoxide dismutase eliminates superoxide radicals and forms hydrogen peroxide, as shown here:



Uric acid may help to preserve the activity of superoxide dismutase [6,7].

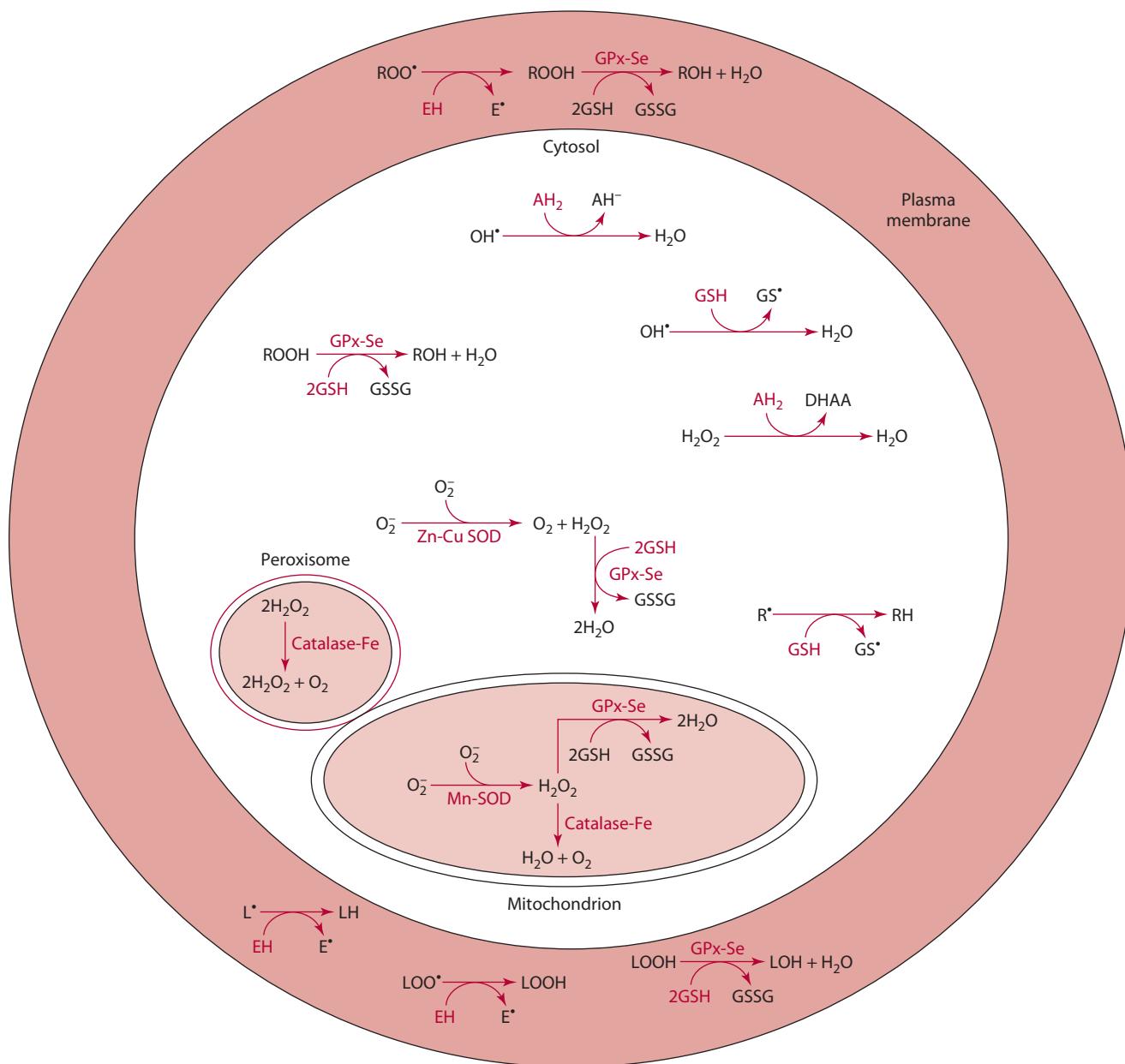
Elimination of Hydrogen Peroxide

Hydrogen peroxide may be disposed of by several mechanisms in cells and tissues. Vitamin C readily reacts with hydrogen peroxide, as do some enzymes. Two enzymes that help to dispose of hydrogen peroxide are glutathione peroxidase and catalase. A third enzyme, myeloperoxidase, uses hydrogen peroxide to generate other radicals needed to help fight bacteria and viruses invading body cells. The role of vitamin C as well as that of each of the enzymes and their antioxidant nutrient cofactors is presented here.

Vitamin C effectively scavenges hydrogen peroxide. Ascorbate (AH_2) reacts with hydrogen peroxide to produce water and dehydroascorbate (DHAA) as shown here:



Glutathione peroxidase, found in the plasma as well as the cytoplasm and mitochondria of cells, is an important enzyme necessary not only for removal of hydrogen peroxide but also for reduction of other peroxides. The enzyme requires the mineral selenium (four atoms) as a cofactor, and activity is impaired if selenium or iron status is poor. Because of selenium's role in glutathione peroxidase, the mineral is considered an antioxidant nutrient. The reaction catalyzed by glutathione peroxidase to remove hydrogen peroxide



Abbreviations

EH = vitamin E	L [•] = carbon-centered lipid radical
AH ₂ = vitamin C	RH = organic nonlipid compound
SOD = superoxide dismutase	R [•] = carbon-centered nonlipid radical
GSH = reduced glutathione	H ₂ O ₂ = hydrogen peroxide
GSSG = oxidized glutathione	ROO [•] = nonlipid peroxy radical
O ₂ ⁻ = superoxide radical	ROOH = nonlipid peroxides
LOO [•] = peroxy radical	LOOH = lipid peroxides
LH = unsaturated fatty acid	

Figure 2 The interactions among selected antioxidant nutrients to prevent cell damage.

requires the tripeptide glutathione (composed of glycine, cysteine, and glutamic acid) in its reduced form (GSH), as shown:



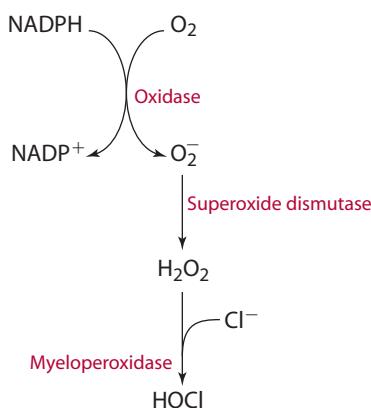
Glutathione is one of many thiols found in both aqueous and lipophilic parts of the body. Thiols are characterized by the presence of sulphydryl residues ($\text{R}-\text{SH}$) and include glutathione, thioredoxin, and lipoic acid, among others. Glutathione (GSH) serves as a reducing agent and, during reactions, becomes oxidized. In the reaction for hydrogen peroxide removal, each of the two glutathione molecules gives up a hydrogen from its sulphydryl group (SH). A radical center is formed on the sulfur atom (GS^*) until two glutathyl radicals join to form a disulfide bond (GSSG).

Catalase is another key enzyme in hydrogen peroxide removal. This enzyme is heme iron-dependent and found mostly in cell peroxisomes (cytoplasmic organelles where lots of hydrogen peroxide is produced during oxidation of very long chain fatty acids, among other molecules). Smaller amounts of the enzyme also are found in the cytosol, mitochondria, and microsomes of cells. Neutrophils and other white blood cells contain fairly high quantities of catalase to dispose of hydrogen peroxide no longer needed in the respiratory burst required for phagocytosis of foreign bacteria, viruses, and fungi. Higher concentrations of hydrogen peroxide are required for catalase activity than for glutathione peroxidase activity. The reaction catalyzed by catalase is shown here:



Thus, accumulation of H_2O_2 is prevented by two enzymes, catalase and glutathione peroxidase. Glutathione peroxidase, because of its dual (mitochondrial and cytosolic) locations in the cell and because of its greater activity at lower hydrogen peroxide concentrations, is thought to be more active than catalase in removing hydrogen peroxide from body cells. Figure 2 shows the complex interaction among components of the oxidant defense system, including the roles of iron-dependent catalase, selenium-dependent glutathione peroxidase, and copper-, zinc-, and manganese-dependent superoxide dismutase (SOD).

Myeloperoxidase is a heme iron–dependent enzyme that uses hydrogen peroxide for the respiratory burst. Remember that the respiratory burst is required to destroy bacteria, viruses, and other harmful substances. Within activated white blood cells, myeloperoxidase is released from the granules into a vacuole that contains the engulfed foreign substance. In this vacuole, the hydrogen peroxide, produced from the superoxide radical, is needed to produce a potent toxic acid, hypochlorous acid (HOCl).

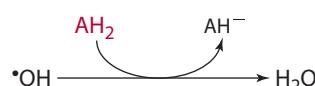


Hypochlorous acid, along with other potent compounds, helps to destroy the foreign bacteria's cell membrane to promote death (lysis) of the foreign substance.

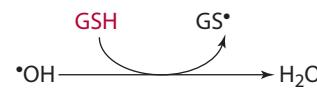
Elimination of Hydroxyl Radicals

Vitamin C and other water-soluble compounds, such as uric acid, various thiols including glutathione and dihydrolipoic acid, and possibly other substances such as metallothionein, serve as defense against hydroxyl radicals. Vitamin E, in contrast, is less effective in eliminating hydroxyl radicals.

Vitamin C can rapidly and effectively react, in aqueous solutions such as blood, with reactive oxygen species before they can initiate oxidative damage. Vitamin C (AH_2) reacts with the hydroxy radical to produce water and the fairly nonreactive semidehydroascorbate radical (AH^-).

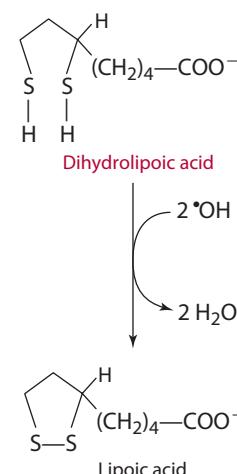


Glutathione may react directly with hydroxyl radicals in aqueous or lipid environments, as shown.



The resulting glutathyl radical (GS^*) typically reacts with another glutathyl radical to produce a disulfide, GSSG.

Dihydrolipoic acid, the reduced form of lipoic acid (also called thioctic acid), functions in the body as a reducing agent to remove hydroxyl radicals (among others) from the environment. Dietary or endogenously generated lipoic acid is reduced in cells to dihydrolipoic acid by dihydrolipamide dehydrogenase, glutathione reductase, or thioredoxin reductase [8]. Dihydrolipoic acid in turn functions in aqueous and lipophilic environments as a reducing agent to eliminate radicals, as shown:



Supplementation with α -lipoic acid has been shown to decrease oxidative stress under some conditions [9,10].

Uric acid and coenzyme Q (also called Q_{10} or ubiquinol) (Figure 3), also may act as reducing agents in aqueous solutions. Both ubiquinol and uric acid may scavenge various free radicals, including $\cdot\text{OH}$.

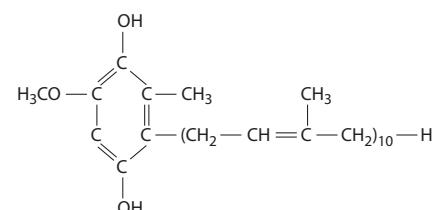


Figure 3 Coenzyme Q or Q_{10} in its reduced form (CoQH_2), also called ubiquinol.

Metallothionein, a protein rich in cysteine residues and thus sulfhydryl groups, is also thought to scavenge hydroxyl radicals [11].

Elimination of Peroxyl, Hydroperoxyl, and Carbon-Centered Radicals, and Lipid Peroxides

Several nutrients and compounds, including vitamin E, carotenoids, manganese, ubiquinol, vitamin C, and glutathione, along with the selenium-dependent enzyme glutathione peroxidase, actively eliminate carbon-centered, peroxy, and hydroperoxy radicals as well as lipid peroxides.

Vitamin E, being lipid-soluble and located near or in membranes, effectively reacts with many radicals, especially carbon-centered radicals and those that initiate peroxidation, such as LOO^* . Specifically, vitamin E donates its phenolic hydrogen on the carbon 6 hydroxyl group. Vitamin E's chromanol ring then stabilizes the unpaired electron.

- Vitamin E (EH) terminates carbon-centered radicals (as shown below) before they abstract further hydrogens from other polyunsaturated fatty acids:



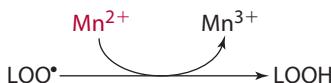
- Vitamin E (EH) prevents peroxidation of polyunsaturated fatty acids by reacting with peroxy radicals (LOO^*) as illustrated in this reaction:



Thus, vitamin E terminates chain-propagation reactions.

Carotenoids such as β -carotene also have the ability to react directly with peroxy radicals involved in lipid peroxidation. β -carotene is thought to carry out this role to a lesser extent than vitamin E and perhaps to function more in the interior of the cell, whereas vitamin E functions on or at the surface.

Some transition metals, such as manganese, may be able to scavenge peroxy radicals as shown here [12]:



In addition to vitamin E, carotenoids, and manganese, ubiquinol has been shown to provide hydrogens to terminate peroxy radicals and thus appears to be a potent antioxidant [13,14]. Ubiquinol

(also called coenzyme Q₁₀ or CoQ₁₀), as CoQH₂, the reduced form of coenzyme Q₁₀, is a small fat-soluble molecule that functions in transporting electrons and ultimately generating ATP in the electron transport chain in the mitochondria. Ubiquinol has been found in small quantities in lipoproteins, where it is thought to be used before vitamin E in the termination of peroxy radicals [13].

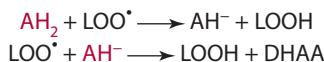


CoQH^{*} may be regenerated into CoQH₂ through the electron transport chain in the mitochondria. Supplementation of humans with 100 or 200 mg ubiquinone₁₀ (CoQ₁₀), the oxidized form of ubiquinol, increased plasma and LDL ubiquinol concentrations and increased LDL resistance to lipid peroxidation [14].

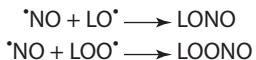
Vitamin C effectively scavenges alkoxyl and peroxy radicals, as shown next.



Both ascorbate (AH₂) and the ascorbate radical (AH⁻) react with peroxy radicals to produce a lipid peroxide and the ascorbate radical or dehydroascorbate (DHAA), respectively, as shown here:



Nitric oxide ($\bullet\text{NO}$) also can act as an antioxidant to terminate lipid alkoxyl (LO^*) and peroxy (LOO^*) radicals as shown here:



Most LOONO homolyzes to produce $\bullet\text{NO}_2 + \text{LO}^*$ and then recombines to produce alkynitrates (LONO_2); however, a small percentage remains as free radicals.

Although eliminating LOO^* is helpful, the often simultaneous generation of lipid peroxides/peroxidized fatty acids (LOOH) can cause problems if the peroxidized fatty acids are within hydrophobic regions of cell membranes. The problems occur because peroxidized fatty acids are polar compounds, and the polarized peroxidized fatty acids, once liberated from the phospholipid in the membrane by the actions of phospholipase A₂, will migrate and destroy the normal architecture of the cell in migrating from the nonpolar region where they are generated.

Glutathione and the selenium-dependent enzyme glutathione peroxidase help to eliminate lipid peroxides.

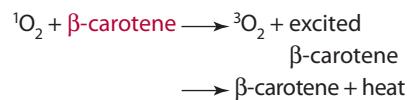
Thiols like glutathione act in both aqueous and lipid environments as antioxidants by providing reducing equivalents (hydrogen ions). Glutathione peroxidase uses glutathione in its reduced form (GSH) and catalyzes the conversion of the peroxides (LOOH) to hydroxy acids, LOH, as follows:



Thioredoxin, which is discussed more on page 425, also can reduce lipid peroxides [15].

Elimination of Singlet Molecular Oxygen

Carotenoids as well as vitamin C and thiols (especially lipoic acid) may quench singlet molecular oxygen. Carotenoids such as β -carotene and lycopene have the ability to directly quench hundreds of singlet oxygen molecules either in solution or in membrane systems. *Quenching* is a process by which electronically excited molecules, such as singlet molecular oxygen, are inactivated [16]. The ability of carotenoids to quench singlet oxygen is attributed to the conjugated double-bond systems within the carotenoid structure. The carotenoids can absorb energy from the singlet oxygen without chemical change to return the "excited" ${}^1\text{O}_2$ to its ground state [4]. Carotenoids then release the energy in the form of heat and thus do not need to be regenerated.



Lycopene has been shown to be more effective than β -carotene in quenching singlet oxygen [17,18].

Regeneration of Antioxidants

When antioxidants provide reducing equivalents, the antioxidants are oxidized. Regenerating the antioxidants is important for further defense against free radicals. It has been estimated that fewer than nine vitamin E molecules exist for every one to two thousand unsaturated fatty acids in cell membrane phospholipids or lipoprotein molecules. Thus, regenerating or recycling vitamins is critical for the vitamin to regain its antioxidant function. This section discusses some of the compounds in the body that recycle antioxidants.

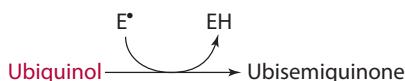
Vitamin E Regeneration

The regeneration of vitamin E is thought to initially require the migration of the vitamin to the membrane surface. At the cell surface, several compounds regenerate vitamin E.

Ascorbate (AH_2) can regenerate α -tocopherol from its radical form (E^*) and, in the process, becomes a radical itself, AH^- , which must be regenerated.



Ubiquinol ($CoQH_2$) also recycles vitamin E, as shown:

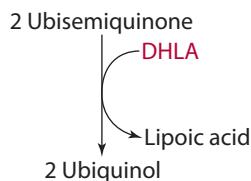


Glutathione in its reduced form (GSH) may donate its hydrogen atom to re-form vitamin E, as follows:

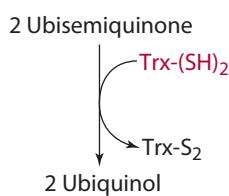


Ubiquinol (Coenzyme QH₂) and Thioredoxin Regeneration

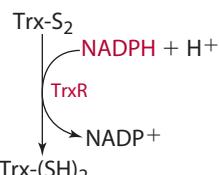
Regeneration of vitamin E by ubiquinol ($CoQH_2$) results in the formation of ubisemiquinone ($CoQH^*$). Ubisemiquinone can be converted back to ubiquinol by dihydrolipoic acid (DHLA), as shown here:



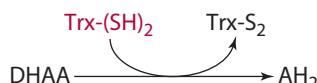
or by the thioredoxin-thioredoxin reductase system. This system, ubiquitous in the body, includes thioredoxin (Trx), a small protein with a dithiol (two sulfhydryl groups -[SH]₂), and the selenium-dependent flavoenzyme (FAD) thioredoxin reductase with a selenocysteine residue at its active site [19,20]. The recycling of ubiquinol is shown here:



Thioredoxin reductase (TrxR) maintains thioredoxin in its reduced form, using reducing equivalents from NADPH as shown here:



Thioredoxin also provides reducing equivalents to dehydroascorbate, as shown here:



Interactions between the vitamin C radicals also permit ascorbate regeneration. Two vitamin C radicals may interact to produce ascorbate (AH_2) and dehydroascorbate (DHAA) as follows:



Antioxidants and Disease

Overproduction of reactive species is thought to contribute to aging and the development of several diseases and conditions including some cancers, heart disease, cataracts, diabetes mellitus complications, and ischemia-reperfusion injury, among others. Nutrients in vitro often demonstrate specific functions or abilities, such as inhibiting lipoprotein cholesterol oxidation or inhibiting cell proliferation or transformation, that are thought to be capable of preventing the development of disease. Despite the promising results of many in vitro studies, the results of supplementation trials in vivo to prevent or treat disease are not consistent and often have shown such use to be detrimental to health [21–37]. Similarly, although many studies have shown that people who either consume diets rich in foods (especially fruits and vegetables) that contain antioxidant nutrients or who have good plasma antioxidant nutrient concentrations have a reduced risk of many diseases or conditions, others do not support such associations [38–69]. New supplementation trials are being conducted, as are in vitro and in vivo studies, to better elucidate the roles and effects of antioxidants. As these studies continue to clarify the roles of the antioxidant nutrients, scientists and other health professionals will continue to reevaluate current recommendations and perhaps develop new guidelines defining optimal levels of nutrients to prevent diseases. However, enjoying a diet rich in fruits, vegetables, and whole grains is always encouraged to help prevent disease and maintain health.

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Web Sites

- <http://www.cancer.gov>
- www.amhr.org
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- www.preventcancer.org
- www.5aday.org

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Calcium

Phosphorus

Magnesium

Sodium

Potassium

Chloride

For each macromineral, the following subtopics (when applicable) are discussed:

Sources

Digestion, Absorption, Transport, and Storage

Functions and Mechanisms of Action

Interactions with Other Nutrients

Excretion

Recommended Dietary Allowance or Adequate Intake

Deficiency

Toxicity

Assessment of Nutriture

PERSPECTIVE

Macrominerals and Hypertension

Osteoporosis and Diet

The importance of minerals in normal nutrition and metabolism cannot be overstated, despite the fact that they constitute only about 4% of total body weight. Their functions are many and varied. They provide the medium essential for normal cellular activity, determine the osmotic properties of body fluids, impart hardness to bones and teeth, and function as obligatory cofactors in metalloenzymes.

Historically, the awareness that minerals are required in normal nutrition evolved from knowledge of the mineral composition of body tissues and fluids. This knowledge has expanded greatly as a result of accumulating improvements in analytical techniques for quantifying minerals.

Macrominerals, also called major minerals or macronutrient elements, are distinguished from the microminerals (Chapter 12) by their occurrence in the body. Using this criterion, various definitions of a macromineral have been expressed, such as the requirement that it constitute at least 0.01% of total body weight or that it occur in a minimum quantity of 5 g in a 60 kg human body. Unfortunately, however, these values clearly are not equivalent. This discrepancy in itself indicates the desirability of a less ambiguous, standard definition, such as required in amounts >100 mg/day.

The major minerals of the human body traditionally include calcium, phosphorus, magnesium, sodium, potassium, and chloride, as shown on the periodic table in Figure 11.1. Because of their importance in maintaining electrolyte balance in body fluids, the macrominerals sodium, chloride, and potassium also are discussed in Chapter 14. Although sulfur is found in the body and is considered a macromineral, the mineral is not discussed as a subsection of this chapter, because the body does not use sulfur alone as a nutrient. Sulfur is found in the body associated structurally with vitamins such as thiamin and biotin, and as part of the sulfur-containing amino acids methionine, cysteine, and taurine. Thus, sulfur is commonly found within proteins, especially those found in skin, hair, and nails.

Table 11.1 provides an overview of the macrominerals, including information on general functions, approximate body content, some enzyme cofactors, deficiency signs, food sources, and recommended intakes. A similar overview of the trace minerals may be found in Chapter 12, Table 12.1. Note the difference in body content between the macro- and microminerals, with the macromineral content of the body ranging from ~35 to 1,400 g, and the micromineral content ranging from <1 mg to ~4 g. In considering the body's mineral content, keep in mind that a pound is equal to 454 g, and an ounce is about 28.4 g.

The major minerals important to human health

1 H Hydrogen																			2 He Helium
3 Lithium Li	4 Beryllium Be																		
11 Sodium Na	12 Magnesium Mg																		
19 Potassium K	20 Calcium Ca	21 Scandium Sc	22 Titanium Ti	23 Vanadium V	24 Chromium Cr	25 Manganese Mn	26 Iron Fe	27 Cobalt Co	28 Nickel Ni	29 Copper Cu	30 Zinc Zn	31 Gallium Ga	32 Germanium Ge	33 Arsenic As	34 Selenium Se	35 Bromine Br	36 Krypton Kr		
37 Rubidium Rb	38 Strontium Sr	39 Yttrium Y	40 Zirconium Zr	41 Niobium Nb	42 Molybdenum Mo	43 Technetium Tc	44 Ruthenium Ru	45 Rhodium Rh	46 Palladium Pd	47 Silver Ag	48 Cadmium Cd	49 Indium In	50 Tin Sn	51 Antimony Sb	52 Tellurium Te	53 Iodine I	54 Xenon Xe		
55 Cesium Cs	56 Barium Ba	71 Lutetium Lu	72 Hafnium Hf	73 Tantalum Ta	74 Tungsten W	75 Rhenium Re	76 Osmium Os	77 Iridium Ir	78 Platinum Pt	79 Gold Au	80 Mercury Hg	81 Thallium Tl	82 Lead Pb	83 Bismuth Bi	84 Polonium Po	85 Astatine At	86 Radon Rn		
87 Francium Fr	88 Radium Ra	103 Lawrencium Lr	104 Rutherfordium Rf	105 Dubnium Db	106 Seaborgium Sg	107 Bohrium Bh	108 Hassium Hs	109 Meitnerium Mt											

57 Lanthanum La	58 Cerium Ce	59 Praseodymium Pr	60 Neodymium Nd	61 Promethium Pm	62 Samarium Sm	63 Europium Eu	64 Gadolinium Gd	65 Terbium Tb	66 Dysprosium Dy	67 Holmium Ho	68 Erbium Er	69 Thulium Tm	70 Ytterbium Yb					
89 Actinium Ac	90 Thorium Th	91 Protactinium Pa	92 Uranium U	93 Neptunium Np	94 Plutonium Pu	95 Americium Am	96 Curium Cm	97 Berkelium Bk	98 Californium Cf	99 Einsteinium Es	100 Fermium Fm	101 Mendelevium Md	102 Nobelium No					

Figure 11.1 The periodic table highlighting the body's major (macro) minerals.

Table 11.1 Macrominerals: Functions, Body Content, Deficiency Symptoms, and Recommended Dietary Allowances (RDAs)

Mineral	Selected Physiological Functions	Approximate Body Content	Selected Enzyme Cofactors	Deficiency Symptoms	Selected Food Sources	RDA/AI
Calcium	Structural component of bones and teeth; role in cellular processes, muscle contraction, blood clotting, enzyme activation	1,400 g	Adenylate, cyclase, kinases, protein kinase, $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase (others, see Table 11.3)	Rickets, osteomalacia, osteoporosis, tetany	Milk, milk products, sardines, clams, oysters, turnip and mustard greens, broccoli, legumes, dried fruits	1,000 mg,* 19–50 years
Chloride	Primary anion; maintains pH balance, enzyme activation, component of gastric hydrochloric acid	105 g		In infants: loss of appetite, failure to thrive, weakness, lethargy, severe hypokalemia, metabolic acidosis	Table salt, seafood, milk, meat, eggs	
Magnesium	Component of bones; role in nerve impulse transmission, protein synthesis; enzyme cofactor	35 g	Hydrolysis and transfer of phosphate groups by phosphokinase; important in numerous ATP-dependent enzyme reactions	Neuromuscular hyperexcitability, muscle weakness, tetany	Nuts, legumes, whole-grain cereals, leafy green vegetables	400 mg males; 310 mg, females; 19–30 years
Phosphorus	Structural component of bone, teeth, cell membranes, phospholipids, nucleic acids, nucleotide coenzymes, ATP-ADP phosphate transferring system in cells, pH regulation	850 g	Activates many enzymes in phosphorylation and dephosphorylation	Neuromuscular, skeletal, hematologic, and cardiac manifestations; rickets, osteomalacia	Meat, poultry, fish, eggs, milk, milk products, nuts, legumes, grains, cereals	700 mg, 19+ years
Potassium	Water, electrolyte, and pH balances; cell membrane transfer	245 g	Pyruvate kinase, Na^+/K^+ -ATPase	Muscular weakness, mental apathy, cardiac arrhythmias, paralysis, bone fragility	Avocado, banana, dried fruits, orange, peach, potatoes, dried beans, tomato, wheat bran, dairy products, eggs	4,700 mg,* 19+ years

Table 11.1 (Continued)

Mineral	Selected Physiological Functions	Approximate Body Content	Selected Enzyme Cofactors	Deficiency Symptoms	Selected Food Sources	RDA/AI
Sodium	Water pH and electrolyte regulation; nerve transmission, muscle contraction	105 g	Na ⁺ /K ⁺ -ATPase	Anorexia, nausea, muscle atrophy, poor growth, weight loss	Table salt, meat, seafood, cheese, milk, bread, vegetables (abundant in most foods except fruits)	1,500 mg,* 19–50 years
Sulfur	Component of sulfur-containing amino acids, lipoic acid, and 2 vitamins (thiamin, biotin)	175 g		Unknown	Protein foods—meat, poultry, fish, eggs, milk, cheese, legumes, nuts	Not established

Note: Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate.

*Adequate intake.

Calcium

Calcium is the most abundant divalent cation of the body, representing about 1.5% to 2% of total body weight, or between ~1,000 and 1,400 g in a 70 kg human. Bones and teeth contain about 99% of the body's calcium. The other 1% is distributed in intra- and extracellular fluids.

SOURCES

The best food sources of calcium include milk and dairy products, especially cheese and yogurt, and selected seafoods, such as salmon and sardines (with bones), clams, and oysters. Milk and yogurt, depending on type, typically provide between 200 and 400 mg calcium/cup, and cheeses generally provide 100 to 200 mg calcium/oz. Seafoods such as sardines (with bones) contain up to 400 mg calcium per 3 oz portion. Selected vegetables, such as turnip and mustard greens, broccoli, cauliflower, and kale also provide relatively high amounts of calcium, ranging from about 30 to 80 mg per half-cup cooked serving. Legumes and legume products, especially tofu (soybean curd) and dried fruits are also relatively rich in calcium. Other foods providing excellent amounts of calcium include those fortified with calcium, such as fruit juices (especially orange juice, which provides up to about 350 mg calcium/serving) and breads. Many forms of calcium supplements also are available, including, for example, calcium carbonate, calcium acetate, calcium lactate, calcium gluconate, calcium citrate, calcium citrate malate, and calcium monophosphate.

Meats, grains, and nuts are relatively poor sources of calcium. Vegetables such as spinach, rhubarb, and swiss chard also are poor sources, because they contain large amounts of oxalic acid, which binds calcium and prevents its absorption, as discussed under “Factors Influencing Absorption.”

DIGESTION, ABSORPTION, AND TRANSPORT

Figure 11.2 presents an overview of calcium digestion, absorption, and transport.

Digestion

Calcium is present in foods and dietary supplements as relatively insoluble salts. Some calcium is released from the salts before absorption. Calcium can be solubilized from most calcium salts in about 1 hour at an acidic pH (as occurs in the stomach). Solubilization does not necessarily ensure better absorption, however, because free calcium can bind to other dietary constituents, limiting its bioavailability.

Absorption

Two main transport processes are responsible for the absorption of calcium, which occurs along the length of the small intestine [1].

■ One of the transport processes, operative primarily in the duodenum and proximal jejunum, is saturable, requires energy, involves a calcium-binding transport protein (CBP; also called calbindin D9k), and is stimulated by calcitriol, also called 1,25-dihydroxycholecalciferol—abbreviated 1,25-(OH)₂D₃—or vitamin D. This transport system is also stimulated by low-calcium (<400 mg) diets, which typically lead to decreased plasma ionized calcium concentrations and an increase in parathyroid hormone (PTH) secretion. Growth, pregnancy, and lactation also increase calcium requirements and improve absorption. Growing children, for example, absorb up to 75% of dietary calcium, in contrast to adults, who average about 30% absorption [2].

Absorption of calcium involves an epithelial calcium channel, specifically transient receptor potential (TRP) V6 (abbreviated TRPV6) channel, and the calcium-binding transport protein calbindin 9K, which binds calcium for transport into the cell. Calcitriol induces the synthesis of calbindin 9K (see Chapter 10, Vitamin D, Functions). With age, however, calcium absorption becomes impaired by decreased renal calcitriol production. High plasma phosphorus concentrations also may diminish calcitriol production in the kidney. Estrogen deficiency at menopause also decreases vitamin D-mediated calcium absorption.

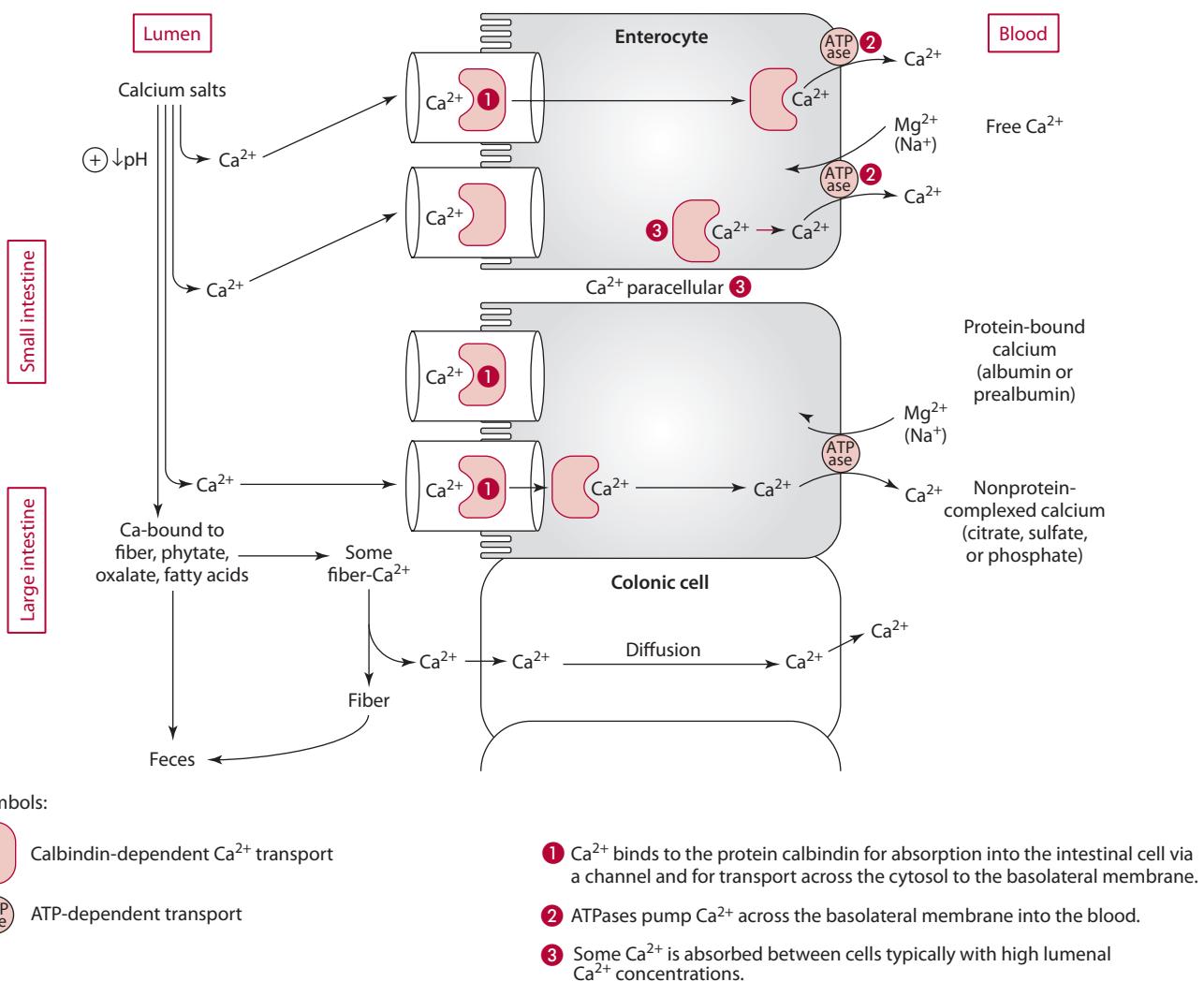


Figure 11.2 Calcium digestion, absorption, and transport.

Following absorption of calcium across the intestinal brush border, calcium is transported across the cytoplasm of the intestinal cell primarily bound to calbindin D9k. This binding minimizes increases in free intracellular calcium, which triggers various events, as discussed later in this section under “Functions and Mechanisms of Action.” Thus, calbindin D9k not only facilitates brush border absorption but also serves as a transport protein to shuttle the calcium across the cytoplasm of the enterocyte to the basolateral (serosal) membrane for extrusion. Other calcium-binding proteins, such as calmodulin, also may facilitate intracellular calcium movement. The extrusion of calcium from the enterocyte into the extracellular fluid requires $\text{Ca}^{2+}\text{-Mg}^{2+}$ ATPase, an enzyme that hydrolyzes ATP and releases energy for pumping Ca^{2+} out of the cell as Mg^{2+} moves in. The $\text{Ca}^{2+}\text{-Mg}^{2+}$ pump is ATP-dependent and is stimulated by vitamin D. Sodium also may be exchanged for Ca^{2+} in the extrusion process at the intestinal basolateral membrane; however, this system is thought to contribute to calcium extrusion from intestinal cells only to a small extent [3].

The second of the two processes for calcium absorption is paracellular. It is a passive process (no carriers or energy needed) that occurs throughout the small intestine, but mostly in the jejunum and ileum. Paracellular absorption is absorption that occurs between cells, rather than through them. The process allows the movement (diffusion) of calcium through normally very tight junctions of the intestinal epithelial cells. Paracellular absorption occurs typically when high concentrations of calcium are present in the lumen and thus a gradient of calcium concentrations between the lumen and the basolateral side. Increases in the concentrations of intracellular calcium ions are thought to mediate the process through a series of reactions to ultimately “open” the junctions between cells to facilitate calcium absorption. Fructose oligosaccharides, inulin, and other nondigestible saccharides have been shown to enhance paracellular calcium absorption [4].

The large intestine also appears to play a role in calcium absorption. Bacteria in the colon may release calcium that

has bound to some fermentable fibers such as pectins. About 4% to 10% (or ~8 mg) of dietary calcium is absorbed by the colon each day; this amount may be higher in people who are absorbing less calcium in the small intestine [2].

Factors Influencing Absorption Several substances are known to enhance or inhibit intestinal calcium absorption (Table 11.2). Vitamin D, as mentioned previously, improves the absorption of calcium. Ingesting food or lactose along with the calcium source appears to improve overall calcium absorption, possibly by improving solubility [5]. The effects of lactose on calcium diffusion, especially in the ileum, are thought to be more pronounced in infants than in adults [5]. Other sugars, sugar alcohols (such as xylitol), and protein also can enhance calcium absorption [5,6].

Phytate (also called phytic acid or myoinositol hexaphosphate and shown later in Figure 11.8) inhibits calcium absorption. Specifically, phytate binds calcium and decreases its availability, especially when present in a phytate:calcium molar ratio >0.2. Some fibers are also thought to bind to calcium and to decrease calcium absorption.

Calcium absorption in the intestine is also inhibited by the presence of oxalate, which chelates the calcium, has a very low solubility (<0.1 mmol/L; optimal solubility is thought to range from about 0.1 to 10.0 mmol/L), and increases fecal calcium excretion. Oxalate is found in a variety of vegetables (e.g., spinach, rhubarb, swiss chard, beets, celery, eggplant, greens, okra, squash), fruits (e.g., currants, strawberries, blackberries, blueberries, gooseberries), nuts (pecans, peanuts), and beverages (tea, Ovaltine, cocoa), among other foods.

Divalent cations, along with other minerals, can compete with calcium for intestinal absorption. For example, magnesium and calcium, both divalent cations, compete with each other for intestinal absorption whenever an excess of either is present in the gastrointestinal tract. Calcium absorption from low-calcium diets (230 mg or less daily) that include supplements of zinc (another divalent cation) also may be impaired [7].

Unabsorbed dietary fatty acids found in significant quantities in the gastrointestinal tract associated with steatorrhea (>7 g of fecal fat per day) can interfere with calcium absorption by forming insoluble calcium “soaps” (calcium–fatty acid complexes) in the lumen of the small intestine. These calcium soaps cannot be absorbed and are excreted in the feces. Steatorrhea is a problem associated with some gastrointestinal tract disorders, such as inflammatory bowel diseases, as well as with disorders affecting the pancreas, such as pancreatitis and cystic fibrosis.

Calcium absorption from calcium supplements varies, depending on the calcium salt. In one study, calcium (250 mg) absorption was $39\% \pm 3\%$ from calcium carbonate, $32\% \pm 4\%$ from calcium acetate, $32\% \pm 4\%$ from calcium lactate, $30\% \pm 3\%$ from calcium citrate, and $27\% \pm 3\%$ from calcium gluconate [1]. Other studies suggest that calcium absorption from chelated forms of calcium such as calcium citrate, calcium

Table 11.2 Interactions between Calcium and Selected Nutrients/Substances

Nutrients/Substances Enhancing Calcium Absorption	Nutrients/Substances Inhibiting Calcium Absorption
Vitamin D	Fiber
Sugars and sugar alcohols	Phytate
Protein	Oxalate
	Excessive divalent cations (Zn, Mg)
	Unabsorbed fatty acids
Nutrients Enhancing Urinary Calcium Excretion	Nutrients Whose Absorption May Be Inhibited by Excessive Calcium
Sodium	Iron
Protein	Fatty acids
Caffeine	

citrate malate, and calcium gluconate is better than that from calcium carbonate [8,9]. Ingesting supplements that provided 250 mg calcium from calcium citrate malate resulted in 35% calcium absorption, and from calcium monophosphate 25% calcium absorption. Calcium carbonate, a widely used form of supplemental calcium, is relatively inexpensive and contains about 40% calcium by weight. Calcium carbonate from fossilized oyster shell or dolomite, however, may be contaminated with aluminum and lead and should not be used [9]. Bone meal preparations may also contain lead and should be avoided. Because the amount of calcium varies among supplements, to obtain 500 mg of calcium a person would need to ingest 5.49 g calcium gluconate, 3.53 g calcium lactate, 2.37 g calcium citrate, 2.16 g calcium acetate, or 1.26 g calcium carbonate [1].

Overall, calcium absorption in adults averages about 30%, the estimate used in deriving recommendations for intakes for adults [2]. Studies report calcium absorption in the range of 20% to 50% from the diet, with most absorption in the range of 20% to 35% from dairy products [1,2,10–12].

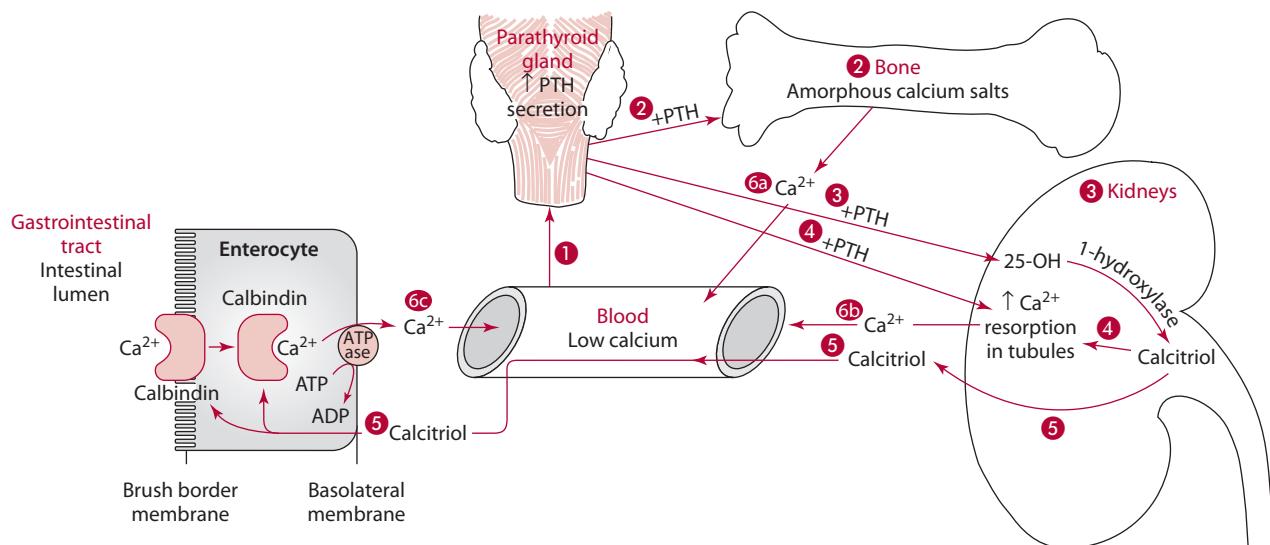
Transport

Calcium is transported in the blood in three forms. Some calcium (~40%) is bound to proteins, mainly albumin and prealbumin. Some calcium (up to ~10%) is complexed with sulfate, phosphate, or citrate. About 50% of calcium is found free (ionized) in the blood.

Regulation of Calcium Concentrations

Calcium concentrations are tightly controlled both intracellularly and extracellularly.

Extracellular Calcium Concentration Regulation Three main hormones are involved in calcium homeostasis in the blood (that is extracellular): PTH, calcitriol, and calcitonin. This section describes each of these hormones and their actions involving calcium; calcitriol and PTH are discussed together. An overview of calcium regulation is shown in Figure 11.3.



- ① Low blood calcium signals the parathyroid gland to release parathyroid hormone (PTH) into the blood.
- ② PTH binds to bone cell receptors and triggers the resorption or breakdown of bone mineral for the release of calcium into the blood.
- ③ PTH acts on the kidneys to synthesize the active form of vitamin D, calcitriol.
- ④ PTH and calcitriol promote the reabsorption of calcium from the kidney and into the blood.
- ⑤ Calcitriol leaves the kidney and travels to the intestine where it promotes the synthesis of calbindin, which facilitates calcium absorption across the brush border membrane of intestinal cells and its transport in the cell cytoplasm.
- ⑥ Calcium enters the blood **a** after release from bone, **b** after release from kidneys, and **c** absorption from intestinal cells.

Figure 11.3 An overview of blood calcium regulation by parathyroid hormone (PTH) and calcitriol (also called $1,25(\text{OH})_2$ vitamin D) in response to low blood calcium concentrations.

- PTH is secreted from the chief cells of the parathyroid gland. PTH secretion is influenced by plasma concentrations of especially calcium but also magnesium. With low plasma calcium concentrations, PTH secretion is increased and calcitonin secretion from the thyroid gland is diminished. Calcium-sensing receptors (CaR) especially on the parathyroid gland and the kidney (tubule) appear to monitor calcium and magnesium concentrations in the blood. Increased concentrations of calcium and magnesium appear to initiate a conformational change in an exterior portion of the receptor that, through a second messenger, signals the parathyroid gland to diminish PTH release.
- PTH, alone or with calcitriol, increases extracellular fluid (plasma) calcium concentrations through interactions with the kidney, intestine, and bone. In the kidney, PTH increases the synthesis of calcitriol from 25-OH vitamin D by 1-hydroxylase. Calcitriol production results in increased renal tubular reabsorption of calcium by calbindin D28k (a vitamin D-dependent calcium transporter found in the kidneys). Calcitriol performs similar functions in the intestine, where it stimulates the synthesis of calbindin D9k. Specifically, calcitriol is thought to interact with vitamin D receptors

in the cytosol of the enterocyte and, following transport to the nucleus, to bind to DNA and induce transcription of genes that code for calbindin D (genomic actions). Calbindin D functions as a calcium-binding protein to promote calcium absorption. Calcitriol also is thought to induce changes in the intestinal cell membranes to enhance calcium absorption (nongenomic actions). In bone, PTH interacts with receptors on osteoblasts (bone-building cells) that signal osteoclasts (bone-breaking cells). Lysosomal proteases and acids in osteoclasts are released and degrade bone (promote resorption), causing calcium to be released from amorphous calcium salts in the bone (found mostly on the bone surface). Calcium pumps, on activation, pump calcium through the bone membrane (periosteum) on the surface of the bone and out into the bone fluid and the blood. Calcitriol also may be involved in this process. Thus, the net effect of PTH and calcitriol is to increase serum calcium concentrations into the normal range.

- Calcitonin is synthesized in the parafollicular cells of the thyroid gland. In contrast to PTH, calcitonin stimulates osteoblasts and lowers serum Ca^{2+} by inhibiting the activity of osteoclasts and thus preventing mobilization

Table 11.3 A Summary of the Effects of Parathyroid Hormone (PTH), Calcitriol, and Calcitonin on Calcium Balance

	PTH	Calcitriol	Calcitonin
Serum calcium	↑	↑	↓
Bone calcium	↓	*	↑
Renal calcium reabsorption	↑	↑	↓
Intestinal calcium absorption	↑	↑	No effect

*Works with PTH.

of Ca^{2+} from bone. Calcitonin also may inhibit production of active vitamin D and diminish renal calcium reabsorption, thus promoting calcium excretion from the body. Table 11.3 summarizes the actions of parathyroid hormone, vitamin D, and calcitonin.

Intracellular Calcium Concentration Regulation Low free Ca^{2+} concentrations (100 nmol/L, or approximately 0.0001 of the concentration in the extracellular fluid) are maintained within the cytoplasm of cells. In response to cell activation by depolarization, neurotransmitters, or hormones, calcium enters the cytoplasm of cells directly from extracellular sites by transmembrane diffusion or by channels (such as voltage-dependent slow channels or agonist-dependent channels). Second messengers also may increase cytosolic calcium levels by stimulating release of calcium from intracellular sites such as the endoplasmic reticulum and the mitochondria. This efflux of organelle-sequestered calcium into the cytoplasm typically requires a pump such as the $\text{Ca}^{2+}, \text{Na}^{+}$ -antiport pump.

Raising the concentration of cytosolic Ca^{2+} allows Ca^{2+} to carry out its cellular functions. Yet, following the release of Ca^{2+} into the cytoplasm, concentrations of calcium are returned within a short time period to their normal levels. To achieve resting concentrations, calcium is exported

from cells by ATP-dependent calcium pumps. These transporters (pumps) require either sodium or magnesium, and they pump Ca^{2+} out of the cell to help maintain low intracellular concentrations. In addition, calcium can be sequestered (stored) in organelles such as the mitochondria, endoplasmic reticulum, nucleus, and vesicles. Calcium, for example, is pumped by a $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase pump into the endoplasmic (or sarcoplasmic) reticulum, whereas a Ca^{2+} pump can drive Ca^{2+} out of the cytoplasm into the mitochondrial matrix for storage until needed by the cell. Within organelles, calcium binds to protein (such as calsequestrin) in the sarcoplasmic reticulum or may complex with phosphate as in the mitochondrion. Figure 11.4 illustrates the mechanisms of cellular control of calcium concentrations.

FUNCTIONS AND MECHANISMS OF ACTION

Calcium functions in the mineralization of bone, of which there are two types: cortical bone and trabecular bone (Figure 11.5). Most bones possess an outer layer of cortical bone that surrounds trabecular bone. Some bones also contain a cavity for bone marrow. Characteristics of cortical and trabecular bone are listed hereafter:

Cortical Bone

- is compact or dense
- represents about 75% to 80% of total bone in the body
- consists of layers of mineralized protein (mostly collagen)
- is found mainly on the surfaces of all bones and the shaft of long bones of the limbs and wrist.

Trabecular Bone

- has a spongy appearance
- represents about 20% to 25% of bone in the body
- consists of an interconnected system of mineralized proteins (mostly collagen)
- is found in relatively high concentrations in the axial skeleton (vertebrae and pelvic region).

Trabecular bone is more active metabolically, with a high turnover rate, and thus is more rapidly depleted of calcium with poor calcium intake than is cortical bone. Despite the differences between cortical and trabecular bone, all bones require mineralization, which involves mainly calcium, phosphorus, and magnesium but also other minerals.

Bone Mineralization

Approximately 99% of total body calcium is found in bones and teeth. About 60% to 66% of the weight of bones is minerals, with the remaining 34% to 40% being water, ground substance, and protein. Minerals, or the mostly inorganic portion of bone, consist largely of calcium and phosphorus, but also include fluoride, magnesium, potassium, sodium, strontium, and hydroxyl groups. Hydroxyl groups make up

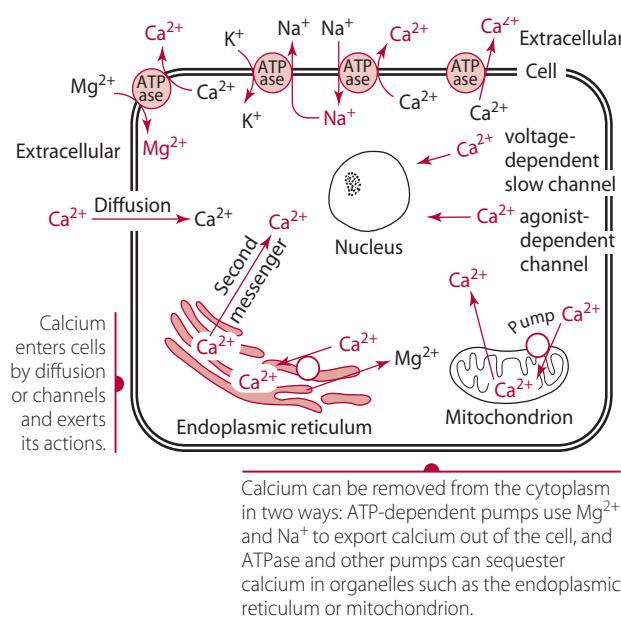


Figure 11.4 Regulation of intracellular calcium concentrations.

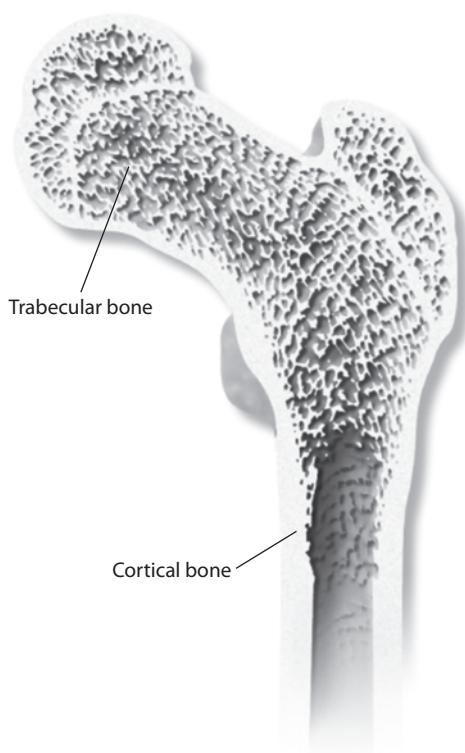


Figure 11.5 Trabecular bone is the lacy network of calcium-containing crystals that fills the interior.

part of **hydroxyapatite**, a crystal lattice–like substance found bound to proteins in bones and teeth. Carbonate is also found in bone, usually associated with calcium, potassium, and sodium. The organic parts of bone contain a range of proteins and ground substance, which form the bone matrix or scaffolding. Proteins in bone include primarily collagen (about 85%–90% of proteins), with smaller amounts of osteonectin, osteopontin, bone sialoprotein, osteocalcin (also called bone Gla protein, abbreviated BGP), and matrix Gla protein (abbreviated MGP). The last two proteins are dependent on vitamin K for carboxylation of their glutamic acid residues and function in calcium binding and matrix modification. Calcium facilitates interactions between proteins or between proteins and phospholipids in cell membranes. Osteonectin is a phosphoprotein that binds both calcium and collagen. Osteopontin binds to both hydroxyapatite and bone cells. Ground substance in bone is made up mostly of glycoproteins and proteoglycans. Glycoproteins consist of proteins covalently bound to typically short chains of carbohydrate. Proteoglycans are similar to glycoproteins but typically are larger, with longer carbohydrate chains; examples include chondroitin 4-sulfate, hyaluronic acid, and keratan sulfate.

Among the three main types of bone cells (osteoblasts, osteocytes, and osteoclasts), osteoblasts are called bone-building cells and originate from the bone marrow. Under the influence of PTH, calcitriol, and estrogen, among other hormones, osteoblasts secrete collagen and

other proteins as well as ground substance—that is, the extracellular matrix (also called the bone matrix osteoid) surrounding the bone cells. As the osteoblasts secrete the proteins and ground substance and mineralization occurs, the osteoblasts become embedded in the proteins and the ground substance matrix. With further embedding in the matrix and morphological changes, osteoblasts become osteocytes. Osteocytes—that is, osteoblasts that have been incorporated into bone matrix—are important to maintaining the integrity of the surrounding bone.

Osteocytes and other bone cells (osteoblasts and osteoclasts) communicate with one another through long processes. The processes are found in channels called canaliculi. A fourth cell type called lining cells also is found in bone. Lining cells are relatively flat cells and form a membrane called the periosteum, which covers the bone surface. This membrane also contains calcium pumps and separates bone from the bone fluid. Thus, bone is not in direct contact with systemic circulation.

During mineralization, calcium, phosphorus, magnesium, and other minerals enter bone fluid from blood and then attach to bone proteins and ground substance. Calcium is first present as Ca^{2+} or as amorphous (noncrystalline or poorly crystalline) calcium forms, such as $\text{Ca}_3(\text{PO}_4)_2$. Other amorphous forms of minerals in bone include carbonate bound to calcium, phosphorus, or magnesium, and, for example, $\text{Ca}_3(\text{PO}_4)_2$ (tricalcium phosphate), $\text{Mg}_3(\text{PO}_4)_2$ (trimagnesium phosphate), and $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (brushite). These salts ultimately are converted to more crystalline compounds such as $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ (octacalcium phosphate) as well as to hydroxyapatite crystals $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Osteoblasts are thought to secrete substances onto the bone surface, which enhances the precipitation or deposition of calcium and other minerals. These substances also break down substances released by osteoclasts that prevent bone mineralization. Whether osteoblasts facilitate the movement of calcium and other minerals from the blood to the bone fluid and then to the bone surface is unclear. The process of calcification and mineralization of the bone matrix has yet to be clearly delineated.

Osteoclasts, another type of bone cell, are large, multinucleated (with about two to ten nuclei) cells that resorb (break down) previously made bone. These cells attach onto a selected area on the bone surface and start the degradation process. Resorption is thought to begin when two proteins, macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor κ B ligand (RANKL), are produced by osteoblasts. These proteins in turn bind to RANK receptors on osteoclast precursor cells to stimulate their proliferation and differentiation. To regulate the process, osteoblasts also produce osteoprotegerin, a protein that binds to RANKL to prevent it from binding to receptors on osteoclastic cells. Osteoclasts contain lysosomes that release acids (such as citric and lactic acids) and enzymes (such as proteases and hydrolases) capable of breaking down the

bone protein and matrix and dissolving amorphous mineral complexes. Osteoclasts respond to PTH, calcitriol, and calcitonin, among other hormones and signalling compounds. Osteoclasts play an important role in increasing blood calcium concentrations to a normal level in times of inadequate calcium intake and contribute to bone fragility and osteoporosis if not balanced by adequate bone formation.

In children and adolescents, skeletal turnover occurs such that formation of bone exceeds resorption of bone. Skeletal turnover continues into adulthood, with peak bone mass occurring in early adulthood. During the fifth decade, bone mass begins to decline. Although the need for calcium in bone modeling is continuous, its greatest benefits in promoting the formation of a sturdy skeletal mass occur during linear bone growth and the years immediately following. The dietary factors involved in osteoporosis, a condition characterized by decreased bone mass, are discussed in the Perspective “Osteoporosis and Diet” at the end of this chapter.

Other Roles

The small amount (1%) of remaining body calcium (that is not associated with bone, or nonosseous) is found both intracellularly within organelles such as the mitochondria, endoplasmic reticulum (sarcoplasmic reticulum in muscle), nucleus, and vesicles and extracellularly in the blood, lymph, and body fluids. Of the calcium in the blood plasma, about 50% is ionized (Ca^{2+}). This ionized calcium is active, which means that the numerous regulatory functions of calcium are performed by <0.5% of the total body calcium. Nonosseous calcium is essential for a number of processes, including, for example, blood clotting, nerve conduction, muscle contraction, enzyme regulation, and membrane permeability. Nerve transmission to muscles, for example, requires calcium. When a nerve impulse is transmitted to the end of a motor neuron, it increases the permeability of the nerve ending to calcium. Calcium then enters the nerve ending and triggers the release of acetylcholine. The acetylcholine diffuses to and binds to receptors on the muscle. This binding in turn triggers increases in sodium and potassium conductance of the membrane and cause an influx of sodium and depolarization. The resulting action potential is conducted along the muscle fiber to initiate contraction.

To achieve many of calcium’s functions, calcium enters the cytosol of the cell, usually in response to a variety of hormones and neurotransmitters. Calcium may also be released into the cytosol from its intracellular storage sites in organelles. The consequential rising of the cytosolic Ca^{2+} concentration allows Ca^{2+} to carry out its functions. Increased free Ca^{2+} concentrations in the cell may affect the cell functions directly or may function through binding to calcium-binding proteins. For example, increased free Ca^{2+} can trigger neutrophils and activate platelet phospholipase A₂, which hydrolyzes fatty acids like arachidonic acid from phospholipids in cell membranes.

The newly released arachidonic acid can in turn be metabolized to form thromboxanes, prostaglandins, or leukotrienes. Phosphodiesterase, which hydrolyzes cyclic AMP (cAMP) to 5'AMP, is also dependent on Ca^{2+} . Cyclic AMP, formed from ATP by adenylate cyclase, activates protein kinases, thereby influencing intermediary metabolism. Calcium also activates protein kinase C, which is involved in a number of cellular processes such as phosphorylating enzymes to activate or inactivate metabolic pathways. Some other enzymes that may be affected either directly by increased free cytosolic Ca^{2+} or through increases in protein-bound Ca^{2+} are listed in Table 11.4. Figure 11.6 shows an example of the release of intracellular calcium from intracellular sites and some of calcium’s actions.

Calcium functions in a variety of processes through interactions with binding proteins. In fact, increased intracellular Ca^{2+} concentrations promote the binding of calcium to any of several calcium-binding proteins. Calmodulin, one example of a calcium-binding protein, appears to be operative in most cells. Calmodulin consists of two similar globular lobes joined by a long helix. Each lobe contains two Ca^{2+} -binding sites, and thus calmodulin in total binds four calcium ions per molecule. Binding of Ca^{2+} activates calmodulin by changing its conformation (Figure 11.7), thereby allowing it to stimulate or interact with a variety of macromolecular processes or enzymes. Some examples of the calmodulin-dependent enzymes include:

- calcineurin, a phosphatase that dephosphorylates and inactivates calcium channels
- myosin light-chain kinase, which phosphorylates the light chain of myosin and, following a sequence of events, causes smooth muscle contraction
- phosphorylase kinase, which activates phosphorylase (the enzyme responsible for glycogenolysis, that is, degrading glycogen to glucose 1-PO₄)
- calcium calmodulin kinases, of which there are several, with several functions

A second example of a calcium-binding protein is troponin C, which is found in skeletal muscle. Skeletal muscle stimulated by nerve impulses (acetylcholine as

Table 11.4 Selected Enzymes Regulated by Calcium and/or Calmodulin

Adenylate cyclase	Myosin kinase
Ca-dependent protein kinase	NAD kinase
Ca/Mg-ATPase	Nitric oxide synthase
Ca/phospholipid-dependent protein kinase	Phospholipase A ₂
Cyclic nucleotide phosphodiesterase	Phosphorylase kinase
Glycerol 3-phosphate dehydrogenase	Pyruvate carboxylase
Glycogen synthase	Pyruvate dehydrogenase
Guanylate cyclase	Pyruvate kinase

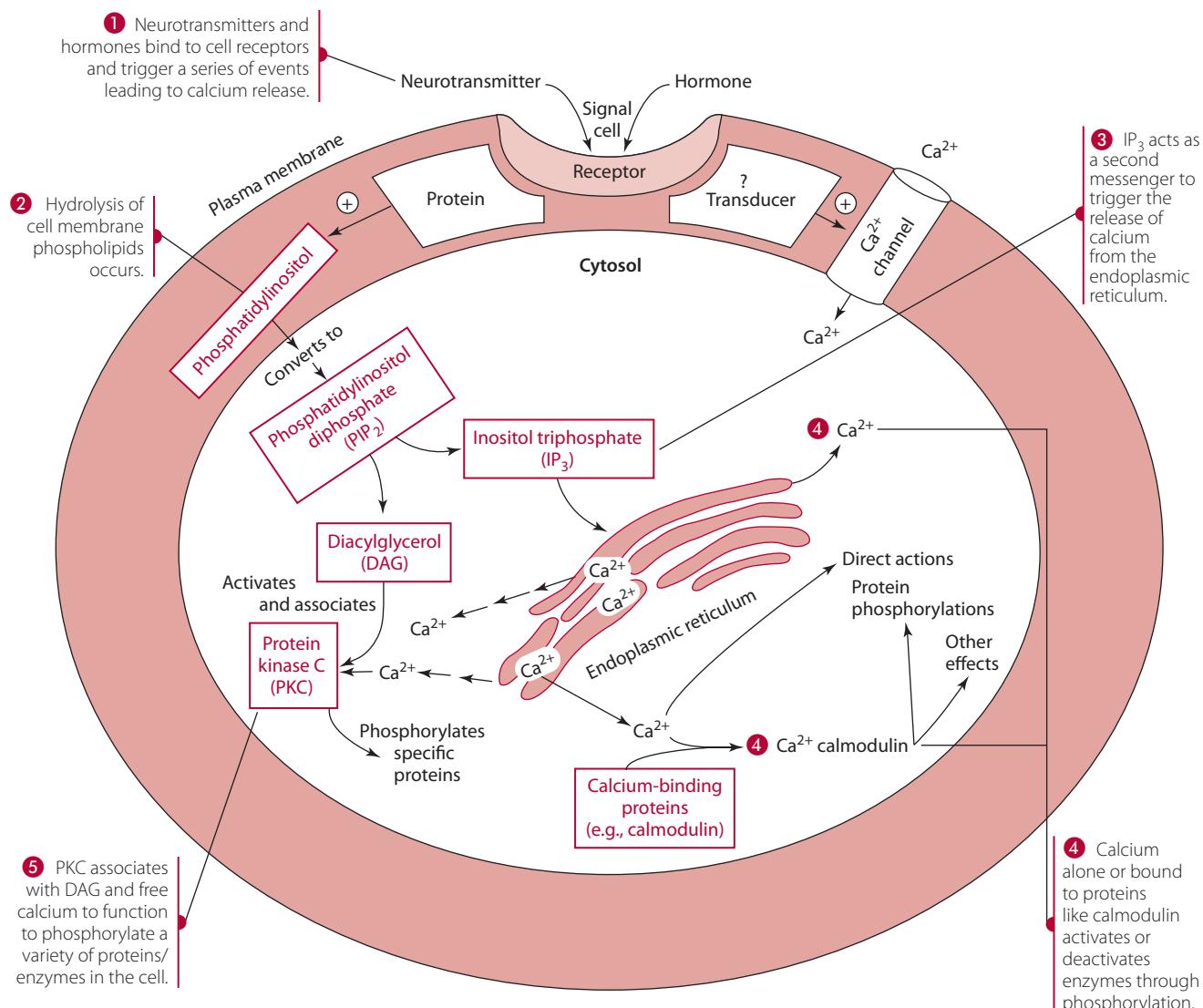


Figure 11.6 Some intracellular actions of calcium.

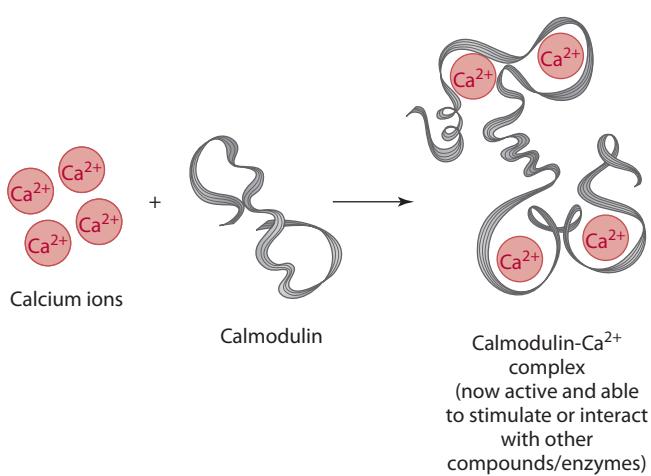


Figure 11.7 Schematic representation of the structural change that occurs in calmodulin following the binding to calcium (Ca²⁺) ions.

neurotransmitter) triggers increased concentrations of calcium. The calcium can then bind to the troponin C, allowing muscle contraction. The structure of troponin C, with its four binding sites for calcium, closely resembles that of calmodulin. Like calmodulin, the conformational change in troponin C caused by Ca²⁺ binding permits an interaction between actin and myosin, resulting in muscle contraction. Once the plasma membrane repolarizes, calcium is pumped back into the sarcoplasmic reticulum, troponin C releases its bound calcium, and myosin and actin no longer interact. Some other calcium- or calmodulin-dependent enzymes are listed in Table 11.4.

INTERACTIONS WITH OTHER NUTRIENTS

Calcium interacts with several nutrients not only at the absorptive surface of the intestinal cell but also within

the body. Some interrelationships between calcium and other nutrients and substances have been discussed in the section on calcium absorption. Additional interactions are discussed here and listed in Table 11.2.

Phosphorus is particularly interesting in that for decades it was thought that certain dietary ratios of calcium to phosphorus should be maintained. Although the need for specific ratios is still believed to be true for infants and children, specific ratios are no longer thought to be as important for adults. Diets low in calcium and high in phosphorus are common in the United States. Prolonged ingestion of diets high in phosphorus and low in calcium may result in a mild secondary hyperparathyroidism. This hyperparathyroidism has been theorized to lead to calcium loss from bone and calcium secretion into the gastrointestinal tract. However, consistent increases in bone resorption have not been shown [13,14]. Also, resorption of bone minerals has been shown to be lower when plasma phosphorus concentrations are high (versus low) at any given PTH concentration [13–20]. Further studies investigating the effects of high-phosphorus, low-calcium diets on bone resorption and acquisition are needed.

The use of calcium supplements to inhibit phosphorus absorption has been known for years. Inhibition of phosphorus absorption is important in the treatment of kidney diseases, because with renal failure, phosphorus excretion is impaired, and plasma phosphorus concentrations rise considerably above normal. For decades, calcium in large amounts (2–3 g per day or in ratios greater than 3 to 1) was prescribed for patients with kidney failure to inhibit phosphorus absorption and thereby help lower plasma phosphorus concentrations. Calcium supplements are no longer typically used for this purpose since calcium phosphate deposition was found to be occurring in soft tissues.

Many interactions among calcium and other nutrients or substances (including protein, sodium, caffeine, alcohol, and boron) occur in the kidney and promote loss of calcium from the body. Dietary protein, for example, promotes urinary calcium losses [21–24]. However, in addition, protein increases calcium absorption and decreases calcium secretion into the gastrointestinal tract to cause no change in total body calcium metabolism [22,25]. Moreover, many protein-containing foods also contain phosphorus, which decreases calcium excretion. Thus, protein does not typically have negative effects on calcium balance. This interaction is also discussed further in the Perspective on osteoporosis at the end of this chapter.

Sodium and calcium excretion are linked in the proximal renal tubule. Sodium consumption of 500 mg per day, for example, can increase urinary calcium excretion by about 10 mg per day [26,27]. See the Perspectives at the end of this chapter for a discussion of sodium as it relates to bone (osteoporosis) and blood pressure.

Caffeine (300–400 mg) produces only small increases urinary calcium (0.25 mmol or 10 mg per day) by reducing

renal reabsorption; however, it may also increase secretion of calcium into the gut, thereby leading to increased endogenous fecal losses [18,28–30]. Caffeine and alcohol intake have each been positively associated with risk of fracture in middle-aged women [30,31]. See the Perspective on osteoporosis at the end of this chapter for further information on caffeine, bone, and osteoporosis.

Other minerals also promote calcium losses from the body. For example, boron supplements (3 mg) when given with magnesium supplements (200 mg) increased urinary calcium losses [32].

Calcium also interacts with some nutrients to inhibit their absorption into the body. Calcium in the form of dietary supplements (providing up to 600 mg calcium in various forms, such as calcium citrate) or in natural food form significantly decreases nonheme iron absorption in a dose-dependent relationship [33–35]. Calcium is thought to affect iron absorption within the enterocyte and not at the brush border. This relationship has been documented in several studies and occurs primarily when calcium and iron are ingested together with food. Calcium supplementation, however, does not appear to negatively affect iron status.

Lead absorption is inversely related to dietary calcium intake [36]. Poor dietary calcium intake also is associated with lead accumulation in blood and organs [37].

Calcium also can diminish the absorption of fatty acids and thus influence serum lipid concentrations and the fatty acid profile of bile. Calcium may work by inhibiting bile acid reabsorption in the ileum, necessitating the use of body cholesterol for the synthesis of additional bile. Calcium also may directly bind the fatty acids in the small intestine to form insoluble “soaps” that are excreted in the feces. Ingestion of calcium carbonate (providing 1,200–3,000 mg calcium) has resulted in significant decreases in total and low-density lipoprotein cholesterol and significant increases in high-density lipoprotein cholesterol [38–41]. Such changes may decrease the risk for heart disease. Calcium supplementation of 2 or 3 g daily decreased chenodeoxycholate concentrations in bile and the lithocholate:deoxycholate ratio in the feces [42]. Such changes are favorable to the colonic environment and may help prevent colon cancer.

EXCRETION

Calcium is excreted in the urine and feces, although up to about 182 mg (average, 60 mg) may be lost daily from the skin, especially with extreme sweating [43]. Most calcium is filtered and reabsorbed by the kidney such that urinary calcium losses range from about 100 to 240 mg per day, with an average of about 170 mg [43,44]. Urinary calcium excretion may be decreased by PTH secretion as well as in the presence of phosphorus, potassium, magnesium, and boron, and it may be increased in the presence of sodium, protein, boron plus magnesium, and caffeine [22–33,45].

Fecal losses of calcium from endogenous sources range from about 45 to 100 mg per day [43] or 3.29 ± 0.83 mmol/day [46]. Phosphorus intake accounts for about 20% of the variance whereby 1 mmol phosphorus ingested increases endogenous calcium losses by 0.037 mmol. Intake of protein (which is often found with phosphorus in foods), however, diminishes endogenous calcium losses in the feces [25,46]. Fecal losses may increase with consumption of phytate and oxalate, and of magnesium in excess, and in people with fat-malabsorbing disorders.

ADEQUATE INTAKE

In 1997, recommendations by the Food and Nutrition Board for adequate intake of calcium were set at 1,000 mg daily for adult men and women age 19–50 years, including women during pregnancy and lactation [2]. For adults age 51 years and older, the calcium recommendations increase to 1,200 mg per day [2]. Additional calcium was not recommended for women on hormone or estrogen replacement during the postmenopausal period, because it does not prevent bone (trabecular) loss that occurs within the first 5 years after menopause [47].

A National Institutes of Health (NIH) panel on osteoporosis also has issued recommendations for calcium intake. Echoing the adequate intake recommendations, the NIH panel recommends that adults ingest 1,000 mg per day; however, for postmenopausal women not treated with estrogen, an intake of 1,500 mg/day is suggested [48]. The NIH panel makes this distinction between women treated and not treated with estrogen because estrogen influences bone mineralization, and without estrogen replacement postmenopausal women experience a rapid loss of bone minerals. A level of 1,500 mg is thought to be associated with maximum calcium retention, and intake $>1,500$ mg daily is thought to represent the threshold amount, beyond which additional intake would not be expected to produce further rises in calcium retention [48,49]. Also recommended by the NIH panel is an intake of 1,500 mg for men age 65 years and older, a target higher than the 1,200 mg suggested for men and women age 51 years and older by the Food and Nutrition Board [2]. The inside covers of this book provide the AIs for calcium for other age groups.

The Food and Drug Administration has approved some health claims related to calcium. One example claim is “Regular exercise and a healthy diet with enough calcium help teen and young adult white and Asian women maintain good bone health and may reduce the risk of osteoporosis later in life” [50]. Another claim added to the aforementioned statement in foods providing 40% or more of the daily value (1,000 mg) states, “Adequate calcium intake is important, but daily intakes above about 2,000 mg are not likely to provide any additional benefit” [50]. Foods citing these claims should not provide

more phosphorus than calcium on a weight-for-weight basis [50].

DEFICIENCY

Inadequate calcium intake, poor calcium absorption, excessive calcium losses, or some combination of these factors contributes to calcium deficiency. Poor calcium intake affects mostly bone and muscle. Rickets occurs in children when the amount of calcium accretion per unit of bone matrix is deficient. Low levels of free ionized Ca^{2+} in the blood (hypocalcemia) may result in **tetany**, a condition characterized by intermittent muscle contractions that fail to relax, especially in muscles of the arms and legs (extremities). Muscle pain, muscle spasms, and paresthesia (numbness or tingling in the hands and feet) also are common signs of tetany. In adults deficient in calcium, osteoporosis—the loss of bone mass (protein matrix and bone minerals)—occurs. This loss of bone increases bone fragility and fracture risk. Osteoporosis and diet are discussed further in a Perspective at the end of the chapter.

Much of the U.S. population, particularly females over 12 years of age, fails to consume the recommended amounts of calcium. Inadequate calcium intake during the period of bone mineralization is a concern because of the high incidence of osteoporosis among elderly women and the significant correlation between present bone density and past calcium intake [2]. Several studies have reported positive effects from consuming adequate dietary calcium, calcium supplements, or both on age-related bone loss [51]. Populations associated with an increased need for calcium include those with high-phytate diets, fat malabsorption, immobilization (which promotes calcium loss from bone), decreased gastrointestinal transit time, and long-term use of thiazide diuretics (which increase calcium excretion in the urine).

In addition to osteoporosis, deficient (long-term) calcium intake also has been associated with the development of hypertension, colon cancer, and obesity or higher body weights. An inverse relationship exists between calcium and blood pressure (as intake of calcium decreases, prevalence of hypertension increases), with a steep slope at calcium intake <600 mg/day [52,53]. The Perspective “Macrominerals and Hypertension” at the end of this chapter addresses this relationship. Calcium also is thought to decrease the risk of colon cancer through its ability to bind (and increase excretion of) bile acids and free fatty acids, which act as promoters of cancer by inducing colon cell hyperproliferation. Colon cancer has been linked with calcium-deficient diets in some but not all studies [2,10,52,54–58]. An adequate intake of calcium (>800 mg/day) is thought to be protective against colon cancer; however, evidence is considered insufficient to recommend the intake of calcium to prevent colon cancer [2]. Low intakes

of calcium and dairy products have also been associated with obesity and high body weights. The modulation of body weight by calcium is under investigation, but it is thought to be related to increased circulating vitamin D and PTH (which occur secondary to low calcium intake and low blood calcium concentrations). In this situation of high blood vitamin D and PTH, calcium entry into adipocytes is greater than normal and the elevation in intracellular calcium in turn promotes gene expression associated with lipogenesis and inhibits lipolysis. Increases in dietary calcium in turn reduce plasma PTH and vitamin D concentrations, reduce calcium uptake into adipocytes, and reduce intracellular calcium concentrations. This “lower calcium environment” in the cell promotes lipolysis and inhibits gene expression enhancing lipogenesis [59–63]. The net result is lower body fat and weight loss with an energy-restricted diet rich in calcium and dairy products.

TOXICITY

Intake of calcium in amounts up to 2,500 mg daily appears to be safe for most people [2]. A tolerable upper intake level of 2,500 mg calcium has been recommended for those age 1 year and older [2].

Milk alkali syndrome has been documented in a few dozen people consuming excessive quantities of calcium in the form of milk and antacids in the treatment of ulcers. The large intake of calcium resulted in hypercalcemia and deposition of calcium in soft tissues, along with systemic alkalosis. Soft tissue calcification typically occurs in patients with renal failure when the plasma calcium concentration times the plasma phosphorus concentration is high. Constipation also can occur when large amounts of calcium are ingested, and for people with idiopathic **hypercalcuria** (urinary calcium levels >4 mg/kg body weight per day) excessive calcium intake may increase the risk of developing calcium-containing kidney stones [2,64].

ASSESSMENT OF NUTRITION

No routine biochemical method appears to assess calcium status accurately. Serum calcium (composed of protein-bound calcium, diffusible calcium complexes, and ionized calcium) is so exquisitely regulated that it usually indicates little about calcium status. Serum calcium concentrations normally range from about 8.5 to 10.5 mg/dL for adults, with slightly higher levels in children. Serum ionized calcium, Ca^{2+} , can reflect alterations in calcium metabolism. Assuming the presence of normal albumin concentrations, the ratio between bound calcium and ionized calcium remains constant. When albumin concentrations are depressed, corrections are needed to adjust for the corresponding decrease that occurs in the protein-bound fraction of calcium. For each 1 g/dL decrease in

serum albumin, serum calcium decreases 0.8 mg/dL. The following equations can be used for estimating protein-bound calcium: Protein-bound calcium (mg/dL) = $0.44 + 0.76 \times \text{albumin (g/dL)}$ or = $0.8 \times (\text{normal albumin} - \text{actual albumin}) + \text{measured calcium (mg/dL)}$.

Bone densitometry can be assessed through computerized tomography (CT) scans. Though less accurate and precise than dual-energy X-ray absorptiometry (discussed below), CT can measure variances in tissue density (such as in vertebral bone). X rays are taken as the person is held in a scanner. Radiation pulses are emitted, collected, and processed to reconstruct the image and calculate bone density.

Neutron activation, in which γ rays are counted following administration of ^{48}Ca into the body and exposure of the body to a low neutron flux, enables assessment of total body calcium content. Results of neutron activation correlate with single-photon absorptiometry, which measures total bone mineral content. Single-photon absorptiometry exposes a portion of a limb, usually the radius (forearm) or os calcis (heel) to radiation. The quantity of bone mineral is inversely proportional to the amount of photon energy transmitted from the bone, as measured by a scintillation counter.

Dual-energy X-ray absorptiometry (abbreviated DEXA or DXA) can be used to measure both body fat and bone mineral content (total and selected sites such as the vertebrae or femur). The procedure involves scanning specific sites at two different energy levels using an X-ray tube. Radiation exposure is very low, and the procedure is relatively quick. Dual-energy X-ray absorptiometry may be used to assess changes in mass over time and is thought to represent the best method for assessing bone mineral density [65]. Further, measurement of bone mass is thought to be the best tool for assessing calcium status. Information on the use of bone mineral density to diagnose osteoporosis is provided in the Perspective “Osteoporosis and Diet” at the end of this chapter.

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Web Sites

www.cfsan.fda.gov
www.nhlbi.nih.gov
www.nationaldairycouncil.org
www.calciuninfo.com
www.consensus.nih.gov

Phosphorus

Among the inorganic elements, phosphorus is second only to calcium in abundance in the body. Approximately 560 to 850 g are present in a 70 kg human, representing about 0.8% to 1.2% of body weight. Of total body phosphorus, about 85% is in the skeleton, 1% is in the blood and body fluids, and the remaining 14% is associated with soft tissue such as muscle. In the body, phosphorus typically is found in combination with other inorganic elements or with organic compounds.

SOURCES

Phosphorus is widely distributed in foods. The best food sources of phosphorus are listed in Table 11.1 and include meat, poultry, fish, eggs, milk, and milk products. Dairy products, for example, contain about 200 to 350 mg phosphorus per serving. An egg has about 100 mg phosphorus. Meats, fish, and poultry provide about 150 to 250 mg phosphorus per 3 oz serving. Nuts, legumes, cereals, and grains also contain phosphorus; however, animal products are superior sources of available phosphorus compared with most plant foods. Coffee and tea also provide small amounts of phosphorus, as do soft drinks. Cola-type soft drinks contain phosphoric acid and, depending

on consumption habits, can contribute substantially to dietary intake. A 12 oz soft drink provides about 25 to 40 mg phosphorus. In addition to dietary sources, phosphate-containing supplements are available commercially, including K-Phos and Neutra-Phos K, which also provide potassium. Such supplements can be helpful for people whose phosphorus stores have been depleted by malnutrition.

Phosphorus is not usually found free in nature. Dietary phosphorus occurs in both an inorganic form and an organic form. In its organic form, phosphorus is bound to a variety of compounds such as proteins, sugars, and lipids. The relative amounts of inorganic and organic phosphorus vary with the type of diet. For example, about one-third of the phosphorus in milk is in the form of inorganic phosphates. Meats contain phosphorus that is largely bound to organic compounds and thus requires hydrolysis for absorption to occur. Over 80% of the phosphorus in grains, such as wheat, rice, and corn, is found as phytate (Figure 11.8; also called phytic acid or myoinositol hexaphosphate). Phosphorus in the form of phytate is also found in beans, legumes, and nuts. The bioavailability of phosphorus from phytate is limited to about 50%, as discussed in the section “Factors Influencing Absorption.”

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Digestion

Regardless of its dietary form, most phosphorus is absorbed in its inorganic form. Organically bound phosphorus is hydrolyzed enzymatically in the lumen of the small intestine and released as inorganic phosphate. Phospholipase C, a zinc-dependent enzyme, for example, hydrolyzes the glycerophosphate bond in phospholipids. Alkaline phosphatase, another zinc-dependent enzyme whose activity is stimulated by calcitriol, functions at the brush border membrane of the enterocyte to free phosphorus from some, but not all, bound forms. For example, it cannot free phytate-bound phosphorus.

Absorption

Phosphorus absorption occurs in its inorganic form throughout the small intestine. However, radiophosphorus perfusion studies suggest that phosphorus absorption occurs primarily in the duodenum and jejunum. About 50% to 70% of dietary phosphorus is absorbed, with absorption

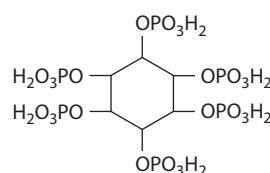


Figure 11.8 Phytic acid (phytate).

from animal products at the upper end of the range, and that from phytate-containing foods at the lower end. Variations in intake have not been shown to affect absorption [1].

Phosphorus absorption appears to occur by two processes:

- a saturable, carrier-mediated active transport system dependent on sodium and enhanced by calcitriol
- a concentration-dependent facilitative diffusion process

Most phosphorus is thought to be absorbed by this latter route. If phosphorus intake is low, then active transport of the mineral increases.

Factors Influencing Absorption A number of factors either positively or negatively influence phosphorus absorption, as shown in Table 11.5. One stimulus of phosphorus absorption is vitamin D as calcitriol, which stimulates absorption in both the duodenum and the jejunum [2].

Several substances inhibit phosphorus absorption. Phytate (Figure 11.8) is the major form of phosphate in grains and legumes. The bioavailability of phosphorus from phytates is poor, because mammalian digestion lacks phytase. Phytase is a phosphate esterase that liberates phosphate from phytic acid. Yeasts in breads possess phytase that can hydrolyze some of the phytates to yield some phosphorus available for absorption. In addition, bacteria in the gastrointestinal tract can liberate some phosphorus from phytates as long as the phytates are not complexed with cations such as calcium, zinc, and iron [3]. When consumed with cations such as Ca^{2+} or Zn^{2+} , phytate forms cation-phytate complexes and prevents these nutrients from being absorbed.

Several minerals, including magnesium, aluminum, and calcium, also impair phosphorus absorption. Phosphorus absorption may be reduced by dietary magnesium, and, conversely, a deficiency of luminal magnesium enhances phosphate absorption. The two minerals are thought to form a complex, $\text{Mg}_3(\text{PO}_4)_2$, within the gastrointestinal tract to render each other unavailable for absorption. Aluminum hydroxide (3 g) given with a meal has been shown to reduce phosphorus absorption from 70% to 35%. Aluminum, magnesium (as hydroxides), and calcium (as carbonate or acetate) are common components of antacids and for years were given in pharmacological doses to bind dietary phosphate in people with **hyperphosphatemia** (high blood phosphorus concentrations) caused by kidney disease.

Table 11.5 Factors Enhancing and Inhibiting Intestinal Phosphorus Absorption

Substances Enhancing Absorption	Substances Inhibiting Absorption
Vitamin D	Phytate Excessive intakes of: Magnesium Calcium Aluminum

Transport and Storage

Phosphorus is quickly absorbed from the intestine and into the blood, appearing in the blood within about an hour after ingestion in animal studies. Phosphorus is found in the blood in both organic and inorganic forms. About 70% of phosphorus is present as organic phosphate, such as that found as phospholipids in lipoproteins. Of the remaining 30% of phosphorus, most (approximately 85%) is as HPO_4^{2-} and H_2PO_4^- with trace amounts of PO_4^{3-} . Some inorganic phosphates are associated with other minerals, such as calcium, magnesium, or sodium [4].

Inorganic phosphorus is sometimes called ultrafilterable phosphate. In adults, plasma inorganic phosphate ranges from about 2.5 to 4.5 mg/dL (0.81 to 1.45 mmol/L). Dietary phosphate, age, time of day, various hormones, and renal function all contribute to the variability of the serum phosphate concentration. Circulating phosphate is in equilibrium with skeletal and cellular inorganic phosphate as well as with that of organic phosphates formed in intermediary metabolism. Phosphorus is found in all cells of the body, though bone and muscle contain most of it. Figure 11.9 provides an overview of phosphorus digestion, absorption, and transport.

FUNCTIONS AND MECHANISMS OF ACTION

Phosphorus has many functions in the body, with involvement in several biologically important compounds. Examples include roles in bone mineralization, energy transfer and storage, nucleic acid formation, cell membrane structure, and acid-base balance. This section briefly discusses each of these roles.

Bone Mineralization

Phosphate is of prime importance in the development of skeletal tissue, which in itself accounts for 85% of body phosphorus. In bone, phosphorus is found in amorphous calcium phosphate forms, including, for example, $\text{Ca}_3(\text{PO}_4)_2$, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Ca}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$, and in more crystalline forms such as hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, which is laid down on collagen in the ossification process of bone formation. In amorphous bone, the ratio of calcium to phosphorus is about 1.3:1, similar to extracellular fluid; however, in crystalline bone, the ratio is about 1.5 to 2.0:1. Phosphorus that is not part of bone is found either in extracellular fluids, such as blood, or in soft tissues. Within cells, phosphorus is the major anion and is involved in a host of processes.

Parathyroid hormone (PTH), calcitriol (1,25-dihydroxycholecalciferol), and calcitonin influence phosphorus balance in the body. Calcitonin promotes the use of phosphorus in bone mineralization. Thus, calcitonin decreases serum phosphorus concentrations (as it does serum calcium). PTH has the opposite effects of calcitonin. PTH (along with

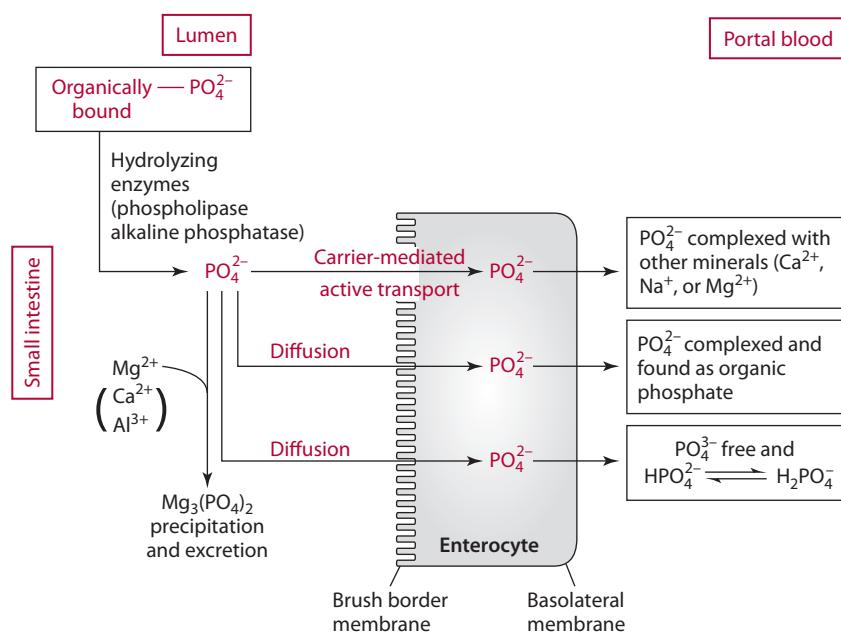


Figure 11.9 Digestion, absorption, and transport of phosphorous.

calcitriol) stimulates resorption of phosphate from bone, possibly through enhanced alkaline phosphatase activity. This action increases serum phosphorus levels; however, PTH also stimulates excretion of phosphorus in urine. The PTH-induced urinary excretion of phosphorus typically is sufficient to override bone resorption of phosphorus so as to effect a net decrease in plasma phosphate. The actions of calcitriol in the intestine stimulate phosphate absorption. The net effect of these hormones is to regulate phosphorus and ensure that the mineral is available to perform its numerous functions in the body, both osseous (bone-related) and nonosseous (i.e., not associated with bone) (discussed in the next section).

Nucleotide/Nucleoside Phosphates

Structural Roles Phosphate is an important component of the nucleic acids DNA and RNA, alternating with pentose sugars to form the linear backbone of these molecules.

Energy Storage and Transfer Phosphorus is of vital importance in intermediary metabolism of the energy nutrients in the form of high-energy phosphate bonds, such as the nucleoside triphosphate adenosine triphosphate (ATP). In addition to its presence in ATP, phosphorus is found in creatine phosphate (also called phosphocreatine). Creatine phosphate is synthesized in muscle from ATP and creatine and can provide energy to muscles as needed (e.g., during exercise) by transferring its PO_4^{2-} to ADP using creatine kinase.

Another nucleoside triphosphate, uridine triphosphate (UTP), activates substances in intermediary metabolism. For example, UTP hydrolysis enables the coupling of uridine monophosphate (UMP) and glucose 1-phosphate to form uridine diphosphate (UDP)-glucose. UDP-glucose is critical for the synthesis of glycogen.

Intracellular Second Messenger Phosphorus as part of cyclic adenosine monophosphate (cAMP), which is derived from ATP, functions as a second messenger to affect cellular metabolism. cAMP, which acts within cells by activating certain protein kinases, is generated in response to the binding of certain hormones to cell receptors. Inositol triphosphate (IP_3) also functions as a second messenger to trigger intracellular calcium release. Its actions are mediated by protein kinases. The role of protein kinases as they function in enzyme activation is discussed next.

Phosphoproteins

Phosphorus also is of vital importance in intermediary metabolism of the energy nutrients through the phosphorylation of different substrates in the body. Protein kinases activated by cAMP, a phosphate-containing second messenger, phosphorylate specific target proteins within the cell, thereby changing cellular activities. Many enzymatic activities, for example, are controlled by alternating phosphorylation or dephosphorylation. An example of the role of phosphorylation and dephosphorylation of enzymes can be found in the discussion of glycogen degradation (page 80).

Structural Roles

Cell membranes are made up in part of lipids, including phospholipids, which (as their name implies) contain phosphorus. Phospholipids, with their polar and nonpolar regions, are important to the bilayer structure of cell membranes. Some examples of phospholipids are phosphatidylcholine, phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine. See Chapter 5 for more information on phospholipids.

Acid-Base Balance

Phosphate also functions in acid-base balance in the body. Within cells, phosphate is the main intracellular buffer. Within the kidney, for example, filtered phosphate reacts with secreted hydrogen ions, releasing sodium ions in the process, as shown here:



This action removes free hydrogen ions and therefore increases pH. The following reaction also increases pH: $\text{HPO}_4^{2-} + \text{H}^+ \longrightarrow \text{H}_2\text{PO}_4^-$. These reactions may be reversed to lower pH.

Oxygen Availability

Phosphate is involved indirectly in oxygen delivery. In red blood cells, synthesis of 2,3-diphosphoglycerate, which influences oxygen release from hemoglobin, requires phosphorus. Decreased 2,3-diphosphoglycerate associated with phosphorus deficiency can diminish release of oxygen to tissues.

EXCRETION

About 67% to 90% of phosphorus is excreted in inorganic form in the urine. The remaining 10% to 33% of phosphorus is excreted in the feces. Unlike calcium, high dietary phosphorus leads to high serum phosphorus, which leads to increased urinary phosphorus excretion. In other words, maintenance of the phosphate balance is achieved largely through renal excretion. The amount of dietary phosphorus and absorbed phosphorus has approximately a linear relationship with urinary phosphorus if the amount of phosphorus filtered is greater than the tubular maximum for phosphorus [1]. The tubular maximum for phosphorus (TmP) is the amount (mmol) of phosphorus reabsorbed per unit time. However, if phosphorus intake and plasma phosphorus concentrations are low, then most filtered phosphorus is reabsorbed. This reabsorption helps maintain the plasma phosphorus concentration. PTH concentrations are inversely related to TmP. Therefore, high levels of PTH decrease TmP and thus increase the amount of phosphorus excreted in the urine. Other hormones that inhibit the tubular reabsorption of phosphorus include estrogen, thyroid hormones, and phosphatonins (also called phosphotoinins, and include various factors such as fibroblast growth factors [FGF]-23, that are known to increase urinary phosphorus excretion).

RECOMMENDED DIETARY ALLOWANCE

An estimated requirement (580 mg/day) for phosphorus was determined based on the relationship between dietary phosphorus intake and plasma phosphorus concentrations as well as a known efficiency of intestinal absorption [5].

A coefficient of variation of 10% was added to the requirement and rounded to establish the recommended intake. The 1997 RDA for phosphorus is 700 mg/day for males and females (including those who are pregnant or lactating) age 19 years and older [5]. These values are slightly lower than the 1989 RDA of 800 mg/day for phosphorus. The inside covers of the book provide the RDAs for phosphorus for other age groups.

DEFICIENCY

Phosphorus deficiency is rare. It is typically confined to people (such as those with renal disease) who are receiving large amounts of antacids containing calcium, magnesium, aluminum, or some combination (which bind phosphorus in the gastrointestinal tract). In addition, people who are malnourished and are being refed enterally through a tube or parenterally (intravenously) without being given additional phosphorus have been known to exhibit phosphate deficiency syndrome. This situation is often called “refeeding syndrome.” Deficiency of phosphorus, usually manifested biochemically by low serum phosphorus concentrations (<1.5 mg/dL), is associated with anorexia, leukocyte dysfunction, reduced cardiac output, decreased diaphragmatic contractility, arrhythmias, skeletal muscle and cardiac myopathy, weakness, neurological problems (ataxia and paresthesia), as well as death. Genetic disorders involving phosphorus include X-linked hypophosphatemia and hypophosphatemic rickets (also called Dent’s syndrome). These disorders result in defects in the reabsorption of phosphorus in the kidneys, and thus cause excessive phosphorus loss from the body.

TOXICITY

Toxicity from phosphorus is rare. Problems have been reported only in infants when calcium:phosphorus ratios are altered significantly in favor of phosphorus. Phosphorus toxicity is characterized predominantly by hypocalcemia and tetany. A tolerable upper intake level of 4 g phosphorus has been recommended for those age 9 to 70 years; after age 70 years, the tolerable level drops to 3 g phosphorus daily [5]. For pregnant and lactating women, the tolerable upper intake levels are 3.5 and 4 g, respectively [5].

ASSESSMENT OF NUTRITION

The assessment of phosphorus nutriture is not a major consideration because deficiency is so rare. Serum phosphorus concentrations and urinary excretion are most often assessed; however, their specificity and sensitivity are low. Serum phosphate concentrations, for example, can be maintained at the expense of tissues.

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Magnesium

Magnesium as a cation in the body ranks fourth in overall abundance, but intracellularly it is second only to potassium. A 70 kg human contains about 35 g magnesium (0.05% of body weight), of which approximately 55% to 60% is located in bone, another 20% to 25% in soft tissues, and about 1% in extracellular fluids.

SOURCES

Magnesium is found in a wide variety of foods and beverages. Beverages rich in magnesium are coffee, tea, and cocoa. Foods (and food components) particularly high in magnesium include nuts, legumes, and whole-grain cereals (especially oats and barley). Beans (such as navy, pinto, kidney, and garbanzo) and black-eyed peas, for example, provide about 40 to 50 mg/half-cup cooked serving. Peanut butter contains about 50 mg magnesium/2 tablespoon serving, and sunflower seeds have about 40 mg magnesium per quarter cup. Whole grain bread and oatmeal each contain about 25 mg magnesium per serving. Spices, seafoods, and green leafy vegetables also provide good amounts of magnesium. Chlorophyll found in the green leafy vegetables contains the magnesium. Spinach, for example, contains about 150 mg magnesium/cup. Seafood like halibut is quite rich in magnesium with over 100 mg per 3 oz serving. Milk and yogurt provide about 30 to 40 mg magnesium per cup. Other particularly good food sources of magnesium are chocolate, blackstrap molasses, corn, peas, carrots, brown rice, and parsley. Tap water also may represent a source of magnesium. Water can be high in magnesium (hard water) or high in sodium (soft water).

Magnesium salts—such as magnesium sulfate ($MgSO_4$, or Epsom salts), magnesium oxide (MgO), magnesium chloride ($MgCl_2$), magnesium lactate, magnesium acetate, magnesium gluconate, and magnesium citrate—are commonly available supplemental forms of the mineral. Slow Mag® (magnesium chloride) and Mag-Tab SR® (magnesium

lactate) tablets provide ~60 to 84 mg magnesium per tablet. Supplements of magnesium are sometimes needed for people with fat malabsorption diseases, such as inflammatory bowel diseases and pancreatic diseases, which cause the malabsorption of not only fat but also magnesium. To maximize absorption, magnesium supplements should not be taken at the same time as other mineral supplements, such as iron.

Food processing and preparation may substantially reduce the magnesium content of some foods. For example, refining whole wheat, which removes the germ and outer layers, can reduce its magnesium content by 80% [1].

ABSORPTION AND TRANSPORT

Absorption

Magnesium absorption occurs throughout the small intestine, mainly in the distal jejunum and ileum [2]. However, the colon also may play a role in absorbing magnesium, especially if disease has interfered with magnesium absorption in the small intestine. Two transport systems are thought to be responsible for magnesium absorption in the small intestine:

- a saturable, carrier-mediated active transporter that operates mostly at low magnesium intakes
- simple diffusion, which is thought to function more with higher intake [3]

The active magnesium transporter is associated with a transient receptor potential (TRP) cation channel called TRPM6. This channel is found on the brush border membrane of small intestinal cells (mostly the duodenum) and on the kidney. The channel's function and thus magnesium absorption is inhibited by high intracellular magnesium concentrations.

About 40% to 60% of magnesium is thought to be absorbed in adults with usual intake [2,4,5]. Absorption declines to about 11% to 35% with magnesium intake ranging from about 550 to 850 mg; magnesium absorption becomes more efficient (up to 75% absorption) when magnesium status is poor or marginal and when magnesium intake is low [5,6]. For example, 65% of magnesium is absorbed with an intake of 36 mg, but only 11% is absorbed with an intake of 973 mg magnesium [3]. Efflux of magnesium out of cells is effected by a sodium- and energy-dependent carrier and possibly a calcium-dependent carrier [7,8]. Figure 11.10 illustrates magnesium absorption and transport.

Factors Influencing Absorption Magnesium absorption may be influenced by a variety of other factors, as outlined in Table 11.6. For example, dietary phytate and nonfermentable fiber have been shown to impair magnesium absorption, but only to a small extent [9–11]. Unabsorbed fatty acids present in high quantities, as with steatorrhea, may bind to magnesium to form soaps. These magnesium-fatty acid

soaps are excreted in the feces. Other minerals, especially calcium and phosphorus, can inhibit magnesium absorption. The inhibition is most apparent when magnesium consumption is low and intake of the other minerals is high. For example, as stated earlier, magnesium and phosphorus can form a complex, $Mg_3(PO_4)_2$, within the gastrointestinal tract to render each other unavailable for absorption.

Some factors are thought to improve magnesium absorption. Vitamin D, in pharmacological doses in some but not all studies, has been shown to increase magnesium absorption by active transport [4,12,13]. Carbohydrates can increase magnesium absorption. For example, fructose enhances absorption by an unidentified mechanism [14]. In addition, lactose consumption by infants or people who are lactose intolerant can improve magnesium absorption [10,15]. The role of dietary protein in magnesium absorption is unclear; some but not all studies suggest improved absorption, whereas some suggest changes in retention [10]. Coatings used on magnesium supplements also affect magnesium absorption. Magnesium absorption from enteric-coated (cellulose acetate phthalate) magnesium supplements (such as Slow-Mag®, containing magnesium chloride) was substantially (67%) less than that from magnesium chloride encapsulated in gelatin [3].

Transport

In the plasma (Figure 11.10), most magnesium (50%–55%) is found free, about 33% is bound to protein, and 13% is complexed with citrate, phosphate, sulfate, or other ions. Of the 33% of magnesium that is protein bound, most (about 30%) is bound to albumin, with the remaining usually bound to globulins. Concentrations of magnesium in the plasma are maintained between about 1.6 and 2.2 mg/dL; however, the homeostatic mechanism of control is unclear. Maintenance of these constant values appears to depend on gastrointestinal absorption, renal excretion, and

Table 11.6 Substances/Nutrients Affecting Intestinal Absorption or Interacting with Magnesium at an Extraintestinal Site

Substances Enhancing Absorption	Substances Inhibiting Absorption	Nutrient Interactions
Vitamin D	Phytate	Calcium
Carbohydrates	Fiber	Phosphorus
Lactose	Excessive unabsorbed fatty acids	Potassium
Fructose		Protein

transmembranous cation flux rather than on hormonal regulation [1]. Several hormones appear to affect but not regulate magnesium metabolism. Parathyroid hormone, for example, increases intestinal magnesium absorption, diminishes renal magnesium excretion, and enhances bone magnesium resorption, thereby raising plasma magnesium concentrations.

FUNCTIONS AND MECHANISMS OF ACTION

About 55% to 60% of magnesium in the body is found associated with bone. Bone magnesium is divided between that found associated with phosphorus and calcium as part of the crystal lattice (~70%) and that found on the surface in an amorphous form (~30%). Bone surface magnesium is thought to represent an exchangeable magnesium pool that is able to maintain serum levels. In contrast, the magnesium in the crystal lattice is probably deposited at the time of bone formation [16]. Magnesium may be present in bone as $Mg(OH)_2$ or $Mg_3(PO_4)_2$, for example.

Magnesium that does not function as part of bone is found in extracellular fluids (1%); in soft tissues, primarily muscle (about 25%) and in organs such as the liver and kidneys [1,4]. Within cells, magnesium is bound to phospholipids as part of cell membranes (plasma, endoplasmic reticulum, and mitochondria), where it may help in membrane stabilization.

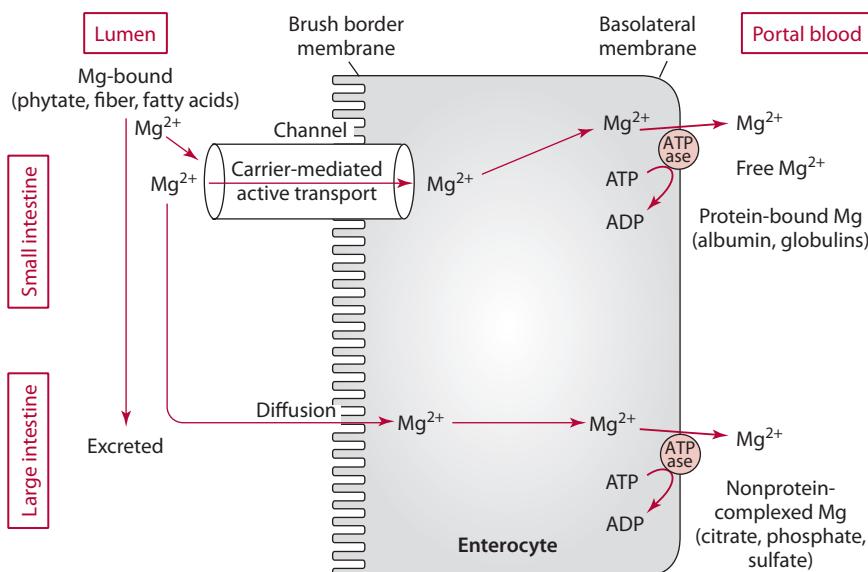


Figure 11.10 Magnesium absorption and transport.

Magnesium is also associated with nucleic acids and protein (enzymes). Magnesium, with an approximate intracellular concentration of 8 to 10 mmol/L, is important for over 300 different enzyme reactions either as a structural cofactor or as an allosteric activator of enzyme activity [4].

Up to about 90% of intracellular magnesium may be associated with ATP or ADP and associated enzymes. Mg-ATP, for example, is used by hexokinase and phosphofructokinase. Mg-ADP is required by phosphoglycerate kinase and pyruvate kinase. In ATP, magnesium binds to phosphate groups, forming a complex that assists in the transfer of ATP phosphate. Figure 11.11 depicts magnesium as a ligand for the phosphate groups of ATP. Protein kinases transfer the γ -phosphate of magnesium ATP to a substrate [16]. Listed here are some of magnesium's roles in the body [16,17]:

- Glycolysis: hexokinase and phosphofructokinase.
- TCA cycle: oxidative decarboxylation.
- Hexose monophosphate shunt: transketolase reaction.
- Creatine phosphate formation: creatine kinase.
- β -oxidation: initiation by thiokinase (acyl CoA synthetase).
- Activities of alkaline phosphatase and pyrophosphatase.
- Nucleic acid synthesis.
- DNA synthesis and degradation, as well as the physical integrity of the DNA helix.
- DNA and RNA transcription.
- Amino acid activation.
- Protein synthesis (e.g., with ribosomal aggregation and binding messenger RNA to ribosome subunits).
- Cardiac and smooth muscle contractility (direct action as well as influence on calcium ion transport and use).
- Vascular reactivity and coagulation (possible role).
- Cyclic adenosine monophosphate (cAMP) formation by adenylate cyclase. Because of its function in forming cAMP, magnesium mediates, in part, the effects of numerous hormones, including parathyroid hormone.
- Ion channel regulation, especially potassium channels.
- Insulin and insulin action.

INTERACTIONS WITH OTHER NUTRIENTS

Magnesium has interrelationships with a number of other nutrients. The first one covered in this section is its interrelationship with calcium. Magnesium is needed for PTH secretion, which is important in calcium homeostasis. High magnesium concentrations, however, appear to inhibit PTH release, similar to calcium. Magnesium also is needed for PTH effects on the bone, kidney, and gastrointestinal tract. The hydroxylation of vitamin D in the liver requires magnesium. Calcium and magnesium

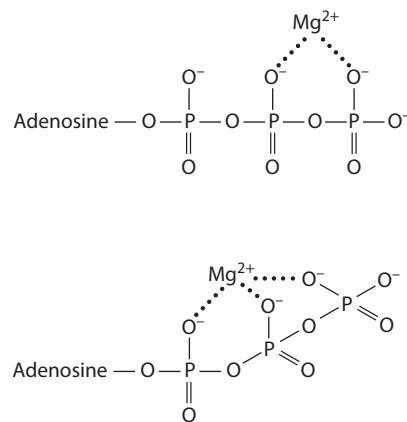


Figure 11.11 Modes by which Mg²⁺ provides stability to ATP.

use overlapping transport systems in the kidney and thus compete in part with each other for reabsorption. Magnesium may mimic calcium by binding to calcium-binding sites and eliciting the appropriate physiological response [17–19]. Magnesium also may cause an alteration in calcium distribution by changing the flux of calcium across the cell membrane or by displacing calcium on its intracellular binding sites. Magnesium may further inhibit release of calcium from the sarcoplasmic reticulum in response to increased influx from extracellular sites and may activate the Ca²⁺-ATPase pump to decrease intracellular Ca²⁺ concentrations [19]. The ratio of calcium to magnesium affects muscle contraction. Magnesium may compete with calcium for nonspecific binding sites on troponin C and myosin [19]. Additional effects of magnesium are seen in the smooth muscles [17,19]. For example, calcium binding initiates acetylcholine release and smooth muscle contraction, and magnesium bound to the calcium sites prevents calcium binding and inhibits contraction [18]. The magnesium-calcium relationship has implications for people with respiratory disease, because increased intracellular calcium promotes bronchial smooth muscle contraction [18]. Magnesium may also influence the process of blood coagulation. In blood coagulation, the actions of calcium and magnesium are antagonistic, with calcium promoting and magnesium inhibiting the process.

Magnesium inhibits phosphorus absorption. As magnesium intake increases, phosphorus absorption decreases. The two minerals are thought to precipitate as Mg₃(PO₄)₂. Magnesium acetate (600 mg), for example, has been shown to reduce phosphorus absorption from about 77% to 34% [3].

A close interrelationship also exists between magnesium and potassium. Magnesium influences the balance between extracellular and intracellular potassium, but its mechanism of action is unclear. Studies have shown that magnesium depletion is associated with increased potassium efflux from cells and subsequent renal excretion but is not associated with changes in the function of Na⁺/K⁺-ATPase, which requires magnesium as an activator [20]. When magnesium and potassium deficiencies coexist, as may occur with some

diuretic drug therapies, magnesium infusions, but not potassium infusions—can normalize muscle potassium.

Finally, dietary protein intake affects magnesium retention. Increasing dietary protein to a marginally adequate level in subjects previously ingesting low magnesium and very low protein diets improved magnesium retention. However, when protein intake was further increased, magnesium retention was decreased [12].

EXCRETION

Most magnesium not retained by the body is excreted through the kidneys. Of the filtered magnesium, about 65% is reabsorbed in the loop of Henle, and another 20% to 30% is reabsorbed in the proximal tubule [5,21]. Thus, only about 5% of the filtered magnesium is excreted in the urine [5,7]. With changes in dietary intake of magnesium, changes in absorption and excretion of magnesium result to maintain, at least in part, homeostasis [5,17,22]. Diuretic medications, as well as protein, alcohol, and caffeine consumption, increase urinary magnesium excretion. In contrast, PTH inhibits magnesium excretion by facilitating its reabsorption in the tubule.

Fecal magnesium concentrations represent unabsorbed magnesium and a small amount of endogenous magnesium. About 25 to 50 mg magnesium from endogenous sources is usually excreted daily in the feces [1]. Magnesium also may be lost in sweat, in amounts estimated to be approximately 15 mg/day [23].

RECOMMENDED DIETARY ALLOWANCE

The RDAs for magnesium published in 1997 suggest 400 mg magnesium for males and 310 mg magnesium for females age 19 to 30 years, and 420 mg magnesium for males and 320 mg magnesium for females age 31 years and older [24]. Requirements used to establish these recommendations, however, are thought to be high by some researchers [25]. Pregnant women age 19 to 30 years should ingest 350 mg, and those age 31 to 50 years should consume 360 mg magnesium [24]. During lactation, women age 19 to 30 years should ingest 310 mg, and those age 31 to 50 years should ingest 320 mg [24]. The inside covers of the book provide the RDAs for magnesium for other age groups.

DEFICIENCY

Deficiency of magnesium or disturbances in magnesium homeostasis is usually associated with the presence of other illnesses. Poor magnesium status may be related to cardiovascular disease, renal disease, diabetes mellitus, toxemia of pregnancy, hypertension, or postsurgical complications [4,19,26,27]. In diabetes, increased urinary magnesium excretion and/or inadequate magnesium absorption appear to be associated with poor glycemic control (hyperglycemia).

Magnesium deficiency in turn further impairs insulin secretion and function. Although the mechanism has not been delineated, inadequate magnesium is hypothesized to inhibit ATP-Mg-dependent reactions and thereby interfere with carbohydrate metabolism. Several studies have examined the relationship between magnesium and type 2 diabetes in various population groups [28–30]. Some studies report and some studies do not report an association between dietary intake and incidence or risk of diabetes. Similar inconsistencies are documented for the relationship between serum magnesium and the development of diabetes [see references 28–30 for a review]. A discussion of magnesium as it relates to hypertension is found in the Perspective on hypertension at the end of this chapter. Other conditions that increase the likelihood of developing a deficiency include excessive vomiting or diarrhea (that is, malabsorptive disorders), protein malnutrition, excessive alcohol use, refeeding syndrome, diuretic use, parathyroid disease, or burns.

Pure magnesium deficiency from inadequate dietary intake has not been reported, but deficiency has been induced under research protocols. Symptoms associated with deficiency or disturbances in balance include nausea, vomiting, anorexia, muscle weakness, spasms and tremors, personality changes, and hallucinations [21]. Changes in cardiovascular and neuromuscular function (such as neuromuscular hyperexcitability) may lead to cardiac arrhythmia and death. Hypomagnesemia, associated with deficiency, represents a plasma magnesium concentration of less than ~1.5 mg/dL and develops within a relatively short time following a magnesium deficit [22,31]. Other biochemical changes include low blood concentrations of not only magnesium but also calcitriol, potassium, and calcium [27,31,32]. Effects on PTH concentrations vary, but concentrations are usually low because parathyroid hormone secretion is diminished; low PTH levels typically result in hypocalcemia (low blood calcium) [31,32]. The observed **hypokalemia** (low blood potassium) results from altered cellular transport systems that maintain the potassium gradient [33]. Calcitriol synthesis may be altered by decreases in PTH secretion or renal resistance to PTH [31]. Bone typically is affected as a result, although bone loss (with inadequate magnesium) is thought to be mediated (in part) by increased release of neuropeptide substance P at nerve endings in bone; substance P enhances osteoclastic bone resorption [32].

TOXICITY

An excessive intake of magnesium is not likely to cause toxicity except in the case of people with impaired renal function. Normal kidneys are able to remove magnesium so rapidly that significant increases in serum concentrations do not occur [23]. Excessive intake of magnesium salts (3–5 g), such as from MgSO₄, may, however, have a cathartic effect, leading to diarrhea and possible dehydration [24]. Other signs, including nausea, flushing, double

vision, slurred speech, and weakness, usually appear at plasma magnesium concentrations of ~9 to 12 mg/dL. Acute magnesium toxicity from excessive intravenous administration of magnesium has resulted in nausea, depression, and paralysis [24]. Muscular paralysis and cardiac or respiratory failure are associated with plasma magnesium concentrations over ~15 mg/dL. A tolerable upper intake level of 350 mg magnesium from nonfood sources has been recommended for people age 9 years and older (including during pregnancy and lactation) [24]. The lowest observed adverse-effect level is 360 mg.

ASSESSMENT OF NUTRITION

Assessment of magnesium status is difficult because extracellular magnesium represents only about 1% of total body magnesium and appears to be homeostatically regulated. Despite low sensitivity and specificity (e.g., normal serum levels may persist despite severe intracellular deficits), serum magnesium concentrations are routinely measured to assess magnesium status [5,23]. When serum magnesium is below normal, an inadequate amount of intracellular magnesium is a certainty. Erythrocyte magnesium concentrations decrease more slowly with magnesium deficiency than plasma or serum concentrations and may reflect longer term magnesium status because of the life span of the red blood cell [5]. Peripheral lymphocyte magnesium concentrations correlate with skeletal and cardiac muscle magnesium content and thus represent a possible indicator of magnesium status [4].

Determining magnesium status more definitively usually involves measurement of renal magnesium excretion, which decreases with magnesium deficiency. Renal magnesium excretion should be measured before and after the administration of an intravenous magnesium load. Decreased excretion determined over two 24-hour periods following administration of the magnesium load indicates deficiency [16].

Alternately, an oral magnesium load test may be used [34]. Normal serum and urinary magnesium concentrations are ~1.6 to 2.6 mg/dL and ~36 to 207 mg per 24 hours, respectively.

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Sodium

Approximately 30% of the ~105 g of sodium in the body (70 kg human) is located on the surface of bone crystals. From that site, it can be released into the bloodstream should **hyponatremia** (low serum sodium) develop. The remainder of the body's sodium is in the extracellular fluid, primarily plasma, and in nerve and muscle tissue. Sodium constitutes about 93% of the cations in the body, making it by far the most abundant member of this family.

SOURCES

The major source of sodium in the diet is added salt in the form of sodium chloride. Sodium comprises 40% by weight of sodium chloride. One teaspoon of salt has 2,300 mg (2.3 g) sodium. Because salt is so extensively used in food processing and manufacturing, processed foods account for an estimated 75% of total sodium consumed. Canned meats and soups, condiments, pickled foods, and traditional snacks (chips, pretzels, crackers, etc.) are particularly high in added salt. Soups and condiments, for example, usually have 400 to 500 mg sodium/serving. Smoked, processed, or cured meats (such as luncheon meats, ham, corned beef, hot dogs), processed cheeses, and canned fish provide about 400 to 800 mg sodium/serving. Moreover, some condiments, like soy sauce, have over 1,000 mg sodium/tablespoon. Naturally occurring sources of sodium such as milk, meat, eggs, and most vegetables furnish only about 10% of consumed sodium. Milk, for example, provides about 120 mg sodium per cup. Meats, poultry, and fish (not processed) provide only about 25 mg sodium per ounce. Breads provide about 160 mg sodium/slice, although quick breads (muffins, biscuits) contain over 300 mg sodium/serving. Fresh vegetables provide typically less than 40 mg sodium per serving, although celery is an exception, containing about 100 mg sodium per cup. In contrast, canned vegetables contain over 200 mg sodium per serving. Instant pasta and rice dishes are exceptionally high in sodium, often providing over 700 mg sodium per serving. Salt added during cooking and at the table provides roughly 15% of total sodium, and water supplies <10%. Depending on the method of assessment, estimates of ingested sodium by Americans range from ~3,000 to 5,000 mg/day.

Terms such as *free*, *very low*, *low*, *reduced*, or *light* in conjunction with sodium on food labels are associated with specific amounts of sodium per serving. For example, *free* means <5 mg sodium per serving, *very low* means <35 mg per serving, and *low* means <140 mg sodium per serving. The term *reduced* or *less* indicates at least 25% less sodium per serving than the appropriate reference food. The term *light* may be used if the food is low in calories and fat and the sodium content has been reduced by at least 50%. The Daily Value on food labels for sodium is 2,400 mg.

Sodium intake has been linked with high blood pressure (hypertension) in some people, as discussed in the Perspective "Macrominerals and Hypertension" at the end of this chapter. The Food and Drug Administration has approved health claims stating "Diets low in sodium may reduce the risk of high blood pressure, a disease associated with many factors" as well as "Development of hypertension or high blood pressure depends on many factors. [This product] can be part of a low-sodium and low-salt diet that might reduce the risk of hypertension or high blood pressure" [1].

ABSORPTION, TRANSPORT, AND FUNCTION

About 95% to 100% of ingested sodium is absorbed, with the remaining 0% to 5% excreted in the feces. Three basic pathways operate in absorption of sodium across the intestinal mucosa brush border membrane. One of these pathways (the Na^+ /glucose cotransport system) functions throughout the small intestine. Another pathway (an electroneutral Na^+ and Cl^- cotransport system) is active in both the small intestine and the proximal portion of the colon. The third pathway (an electrogenic sodium absorption mechanism) operates principally in the colon.

The Na^+ /glucose cotransport system involves a carrier on the brush border (apical) membrane of the small intestine. Na^+ and glucose bind to the carrier, which shuttles them from the outer surface to the inner surface of the cell membrane. There both are released before the carrier returns to the outer surface. Absorbed Na^+ is then pumped out across the basolateral (serosal) membrane by the Na^+/K^+ -ATPase pump, while the glucose diffuses across the membrane by a facilitated transport pathway. The Na^+ gradient created by the Na^+/K^+ -ATPase pump provides the energy needed to maintain the absorptive direction of the ion. Cotransport of Na^+ by this mechanism also can occur with solutes other than glucose, including amino acids, di- and tripeptides, and many B vitamins.

The electroneutral Na^+ and Cl^- cotransport mechanism has been proposed because of the observation that a significant portion of sodium uptake requires the presence of chloride, and vice versa [2]. Precisely how this system functions has not yet been established. However, the cotransport is believed to be composed of Na^+/H^+ exchange working in concert with a $\text{Cl}^-/\text{HCO}_3^-$ mechanism [3]. The mechanism allows the entrance of both Na^+ and Cl^- into the cell, where they are exchanged for H^+ and HCO_3^- . Protons and HCO_3^- are produced within the cell by the action of carbonic anhydrase on CO_2 . Absorbed Na^+ is pumped across the basolateral membrane by the Na^+/K^+ -ATPase pump, followed by Cl^- , which crosses by diffusion.

The colonic mechanism is called an electrogenic sodium absorption mechanism because the absorbed sodium ion is the only ion moving transcellularly, allowing its transport

to be monitored. It enters the luminal membrane of the colonic mucosal cell through Na^+ -conducting pathways called Na^+ channels, diffusing inwardly by the downhill concentration gradient of the ion. The absorbed sodium is accompanied by water and anions, resulting in net water and electrolyte movement from the luminal side to the bloodstream side of the colon epithelium. It is pumped out across the basolateral membrane on the bloodstream side of the cell by the Na^+/K^+ -ATPase pump.

All three of these mechanisms are depicted schematically in Figure 11.12. Note that the common driving force for sodium absorption in all the processes is the inwardly directed gradient maintained by the basolateral Na^+ pump.

Once absorbed into the body, sodium is transported freely in the blood. Serum sodium concentrations, as well as those of potassium and chloride, are maintained within a fairly narrow range (~135 to 145 mEq/L) by several hormones, including antidiuretic hormone (ADH) or vasopressin, aldosterone, atrial natriuretic hormone, renin, and angiotensin II.

Within the body, sodium plays important roles in the maintenance of fluid balance, nerve transmission/impulse conduction, and muscle contraction. Although proteins play a role in fluid balance, they normally remain either intracellular or extracellular. Sodium, potassium, and chloride therefore display the most movement across cell membranes to maintain osmotic pressure and thus fluid balance. Sodium's roles in nerve transmission and muscle contraction involve sodium as part of the Na^+/K^+ -ATPase pump found in the plasma membrane of cells. With the exchange of sodium for potassium and the hydrolysis of ATP, an electrochemical potential gradient generates nerve or impulse conduction.

INTERACTIONS WITH OTHER NUTRIENTS

It has long been recognized (since before 1940) that dietary sodium intake affects urinary calcium excretion. Studies have shown that accompanying the calciuria are decreases in fecal calcium excretion and increased calcium absorption. Such calcium-elevating effects partially offset the urinary calcium losses [4–6]. The sodium-calcium interaction and its possible association with osteoporosis are presented in more detail in the Perspective “Osteoporosis and Diet” at the end of this chapter.

EXCRETION

Because nearly all the ingested sodium is absorbed, much larger amounts are absorbed than are required by the body. Sodium in excess of that needed by the body is excreted primarily by the kidneys. Sodium losses also take place through the skin by sweating. Under conditions of moderate temperature and level of exercise, sodium losses by sweating are small. However, because the

sodium content of sweat is about 50 mEq/L, it can be reasoned that conditions of high temperature or sustained vigorous exercise can account for significant losses. Renal excretion and retention of sodium are under the control of aldosterone, which promotes the retention (reabsorption) of sodium and the excretion of potassium. The hormone is released from the adrenal cortex in response to low sodium or, more important, high potassium concentrations. The renal regulation of sodium, as well as of potassium and chloride, is presented in greater detail in Chapter 14.

DEFICIENCY

Dietary deficiencies of sodium do not normally occur because of the abundance of the mineral across a broad spectrum of foods. Serum concentrations of sodium normally are regulated within the range of ~135 to 145 mEq/L. However, with excessive sweating involving a loss of more than about 3% of total body weight, deficiencies of sodium have been reported. Symptoms include muscle cramps, nausea, vomiting, dizziness, shock, and coma.

ADEQUATE INTAKE AND ASSESSMENT OF NUTRITION

The National Research Council has suggested an adequate intake of 1,500 mg (65 mmol) sodium (or 3.8 g salt) for adults per day [7]. The minimum amount of sodium needed to replace losses (with no sweat and maximal adaptation) is estimated at about 180 mg (8 mmol); however, this amount is not thought to represent the requirement [7]. A tolerable upper intake level of 2,300 mg (100 mmol) sodium for adults per day has been established [7]. Given that the average person consumes between 3–5 g sodium per day, most people greatly exceed these intake recommendations. Interestingly, patients with various health conditions such as hypertension and kidney disease, are put on sodium-restricted diets of 2 g (that is higher than the current adequate intake recommendations). Such diets typically restrict intake of foods high in sodium (i.e., canned soups; canned and brined vegetables; smoked, cured, and processed meats, fish, and cheeses; quick breads; salted snack foods; prepared package frozen foods; instant rices, pasta, and potato dishes; and condiments). Additional recommendations for sodium for other age groups are provided on the inside covers of the book. The Perspective at the end of this chapter provides further information on sodium as it relates to hypertension and osteoporosis.

Sodium is measured routinely in clinical laboratories, especially to determine electrolyte balance (see Chapter 14). Sodium in the serum and other biological fluids is usually quantified by the technique of ion-selective electrode **potentiometry**. This method measures Na^+ in the same

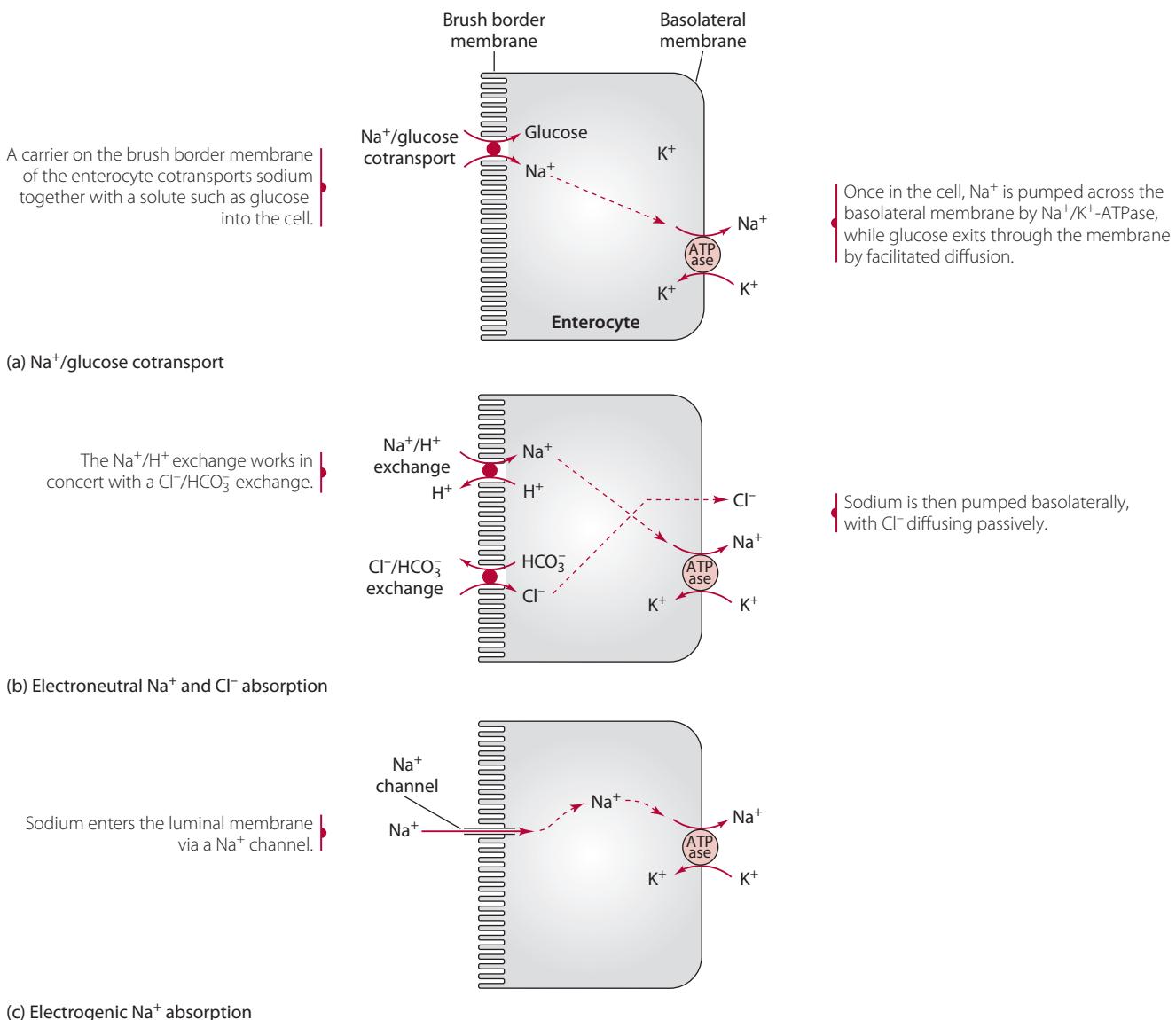


Figure 11.12 Absorption mechanisms for sodium in the intestine (a–c).

way a pH meter measures protons. Twenty-four hour urinary sodium excretion is most often used as a reflection of sodium intake.

Potassium

Potassium is the major intracellular cation. In fact, in contrast to sodium, about 95% to 98% of the body's potassium is found within body cells. Potassium constitutes up to ~0.35% of total body weight, or up to ~245 g in a 70 kg human.

SOURCES

Potassium is widespread in the diet and is especially abundant in unprocessed foods, which provide potassium along with anions like phosphate and citrate (note that

citrate is thought to be important, because it can serve as a precursor to bicarbonate in the body for acid-base balance). Foods exceptionally rich in potassium (usually greater than 300 mg per serving) include some fruits like prune juice, bananas, cantaloupe, honeydew melon, mango, and papaya, and some vegetables (avocados, winter squash, leafy green vegetables, and yams). Other good sources of potassium, containing between about 200–300 mg potassium per serving, are legumes, nuts and seeds, peanut butter, selected vegetables (such as potatoes, asparagus, mushrooms, and okra), and fruits (like orange juice, grapefruit juice, peaches, pears, kiwi, and nectarines). Milk and yogurt also provide potassium—about 300 mg per cup. In addition to unprocessed sources, salt substitutes often contain potassium in place of sodium.

Diets high in potassium are associated with lower blood pressure; this topic is discussed further in the Perspective “Macrominerals and Hypertension” at the end

of this chapter. The Food and Drug Administration has approved the health claim “Diets containing foods that are good sources of potassium and low in sodium may reduce the risk of high blood pressure and stroke” [1]. To use this claim, a food must contain at least 350 mg (10% daily value) potassium; must have ≤ 140 mg sodium, ≤ 3 g total fat, ≤ 1 g saturated fat, and ≤ 20 mg cholesterol; and must provide $\leq 15\%$ of energy (kcal) from saturated fat [1]. For food labeling purposes, to be considered a “rich,” “excellent,” or “high” source of potassium (any nutrient), a food must contain 20% or more of the daily value; to be a “good” source of potassium, the food must contain 10% to 19% of the daily value [1].

ABSORPTION, TRANSPORT, AND FUNCTION

The mechanisms by which potassium is absorbed from the gastrointestinal tract are not as clearly understood as the mechanisms of sodium absorption. Over 85% of ingested potassium is absorbed, although the exact sites along the small intestine at which absorption takes place have not been precisely identified [8–10]. In addition to being absorbed in the small intestine, K^+ may be absorbed across the colonic mucosal cells. Depending on concentration, potassium is thought to be absorbed by passive diffusion or by a K^+/H^+ -ATPase pump. This pump exchanges intracellular H^+ for luminal K^+ . Alternatively, K^+ may enter the cell through apical (brush border) membrane channels that also serve as secretory pathways.

To enter the blood, the K^+ accumulated in the intestinal cell diffuses across the basolateral membrane through the K^+ channel. Uptake of potassium into nonintestinal cells occurs by active transport. Intracellular potassium concentrations also are maintained by Na^+/K^+ -ATPase pumps, which are stimulated by hormones, especially insulin and some catecholamines. Potassium, however, also influences insulin with hypokalemia reducing insulin secretion.

Potassium influences the contractility of smooth, skeletal, and cardiac muscle and profoundly affects the excitability of nerve tissue. It is also important in maintaining electrolyte and pH balance.

INTERACTIONS WITH OTHER NUTRIENTS

Like sodium, potassium has an effect on the urinary excretion of calcium. However, its effect is opposite to that of sodium, in that it decreases calcium excretion, and sodium increases it. Replacement of some of the NaCl in the diet with KCl to reduce the amount of NaCl consumed has been shown to reduce urinary calcium excretion [11]. The addition of potassium citrate (90 mmol/day) to a diet high in salt (225 mmol/day) can prevent the normal increase in urinary calcium associated with the high salt diet [12]. Moreover, this addition of potassium citrate significantly decreases markers of bone resorption that have

been associated with a high salt intake in postmenopausal women [12]. A discussion of potassium and bone is found in the Perspective at the end of this chapter.

EXCRETION

Most potassium (up to ~90%) is excreted from the body by the kidneys, with only small amounts excreted in the feces. As with sodium, potassium balance is achieved largely through the kidneys, with aldosterone being the major regulatory hormone. Aldosterone acts reciprocally on sodium and potassium. Although it stimulates the reabsorption of sodium in the kidney tubules, aldosterone accelerates the excretion of potassium. Renal control of potassium is discussed further in Chapter 14. Thiazide and loop diuretics (medications used to treat high blood pressure) increase urinary potassium excretion; many people taking such medications require potassium supplements to maintain normal plasma potassium concentrations.

DEFICIENCY AND TOXICITY

Hyperkalemia (abnormally high serum potassium concentration) is toxic, resulting in severe cardiac arrhythmias and even cardiac arrest. Producing hyperkalemia by dietary means is nearly impossible in a person with normal circulation and renal function because of potassium’s delicate control within a narrow concentration range. Similarly, hypokalemia (abnormally low serum potassium $<\sim 3.5$ mmol/L) does not occur by dietary deficiency because of the abundance of potassium in common foods. Hypokalemia is associated with cardiac arrhythmias, muscular weakness, nervous irritability, hypercalcemia, glucose intolerance, and mental disorientation and can result from profound fluid loss, such as the losses that occur with severe vomiting and diarrhea or with use of some diuretic medications. Hypokalemia also may occur as part of refeeding syndrome, which occurs when malnourished people are being refed (usually intravenously or through a tube) a diet lacking enough supplemental potassium to replace that lost from the cells during the starvation period and needed for the body as it synthesizes new lean body mass. A moderate deficiency of potassium (without hypokalemia) is associated with elevations in blood pressure, increased urinary calcium excretion, and abnormal bone turnover (increased bone resorption and decreased bone formation). The Perspectives at the end of the chapter provide further information on these topics.

ADEQUATE INTAKE AND ASSESSMENT OF NUTRITION

The National Research Council has suggested an adequate intake of 4,700 mg (120 mmol) potassium per day for adults [7]. Additional recommendations for potassium for

other population groups are provided on the inside covers of the book. The potassium intake of most Americans (about 3,300 mg) does not meet recommendations, and even consuming a diet rich in fruits and vegetables, such as the DASH diet used to treat hypertension, can still leave a person just short of meeting recommendations. Thus, it takes careful diet planning to meet the current recommendations for potassium. No tolerable upper intake level has been established for potassium from foods; however, note that if potassium supplements are used, it should be done only under the recommendations and monitoring of medical personnel, because too much (or too little) potassium in the blood can be lethal.

Potassium status is typically assessed based on plasma potassium concentrations. The normal serum concentration of potassium, as K^+ , is ~3.5 to 5.0 mEq/L. Serum potassium levels, like those of sodium, are determined primarily by ion-selective electrode potentiometry.

Chloride

Chloride is the most abundant anion in the extracellular fluid, with approximately 88% of chloride found in extracellular fluid and just 12% intracellular. Its negative charge neutralizes the positive charge of the sodium ions with which it is usually associated. In this respect, it is of great importance in maintaining electrolyte balance. Total body chloride content is similar to that of sodium, representing about 0.15% of body weight, or about 105 g in a 70 kg human.

SOURCES

Nearly all the chloride consumed in the diet is associated with sodium in the form of sodium chloride, or salt. Salt, which is about 60% chloride, is abundant in a large number of foods, particularly in snack items and processed foods. Chloride also is found in eggs, fresh meats, and seafood. The average adult consumes an estimated 50 to 200 mEq chloride/day.

ABSORPTION, TRANSPORT, AND SECRETION

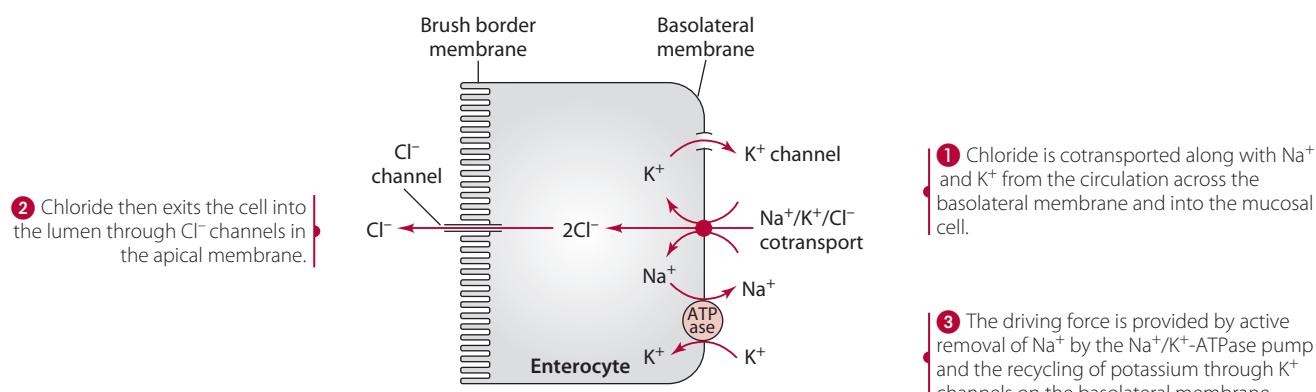
Chloride is almost completely absorbed in the small intestine. Its absorption closely follows that of sodium in the establishment and maintenance of electrical neutrality. The absorptive mechanisms, however, generally are different. For example, in the Na^+ -glucose cotransport system (described in the section on sodium), chloride follows the actively absorbed Na^+ passively through a so-called paracellular, or tight junction, pathway. The absorbed Na^+ creates an electrical gradient that provides the energy for the accompanying inward diffusion of Cl^- . The

electroneutral Na^+/Cl^- cotransport absorption system also contributes to the movement of chloride into the mucosal cells, although the relative contribution of this system to total chloride absorption is not well established. Sodium absorbed by the electrogenic Na^+ absorption mechanism also is accompanied by chloride, which follows the absorbed sodium passively (paracellularly) to maintain electrical neutrality. Clearly, regardless of which absorptive mechanism is functioning, wherever sodium goes, chloride cannot be far behind!

Secretory mechanisms for the electrolytes throughout the gastrointestinal tract center on chloride, which is the major secretory product of the stomach and the rest of the gastrointestinal tract. The well-defined mechanism is an electrogenic Cl^- secretion. Cl^- is the only ion actively secreted by the epithelium, and its movement can be monitored by changes in electrical potentials. Cells take up chloride from the blood across the basolateral membrane by way of an $Na^+/K^+/Cl^-$ cotransport pathway. An appropriate gradient is set up by the Na^+/K^+ -ATPase pump, which maintains a low concentration of intracellular sodium. Potassium channels on the basolateral membrane allow potassium recycling out of the cell. Chloride accumulating in the cell exits through the brush border membrane into the lumen through the Cl^- channels. Figure 11.13 illustrates the chloride secretory mechanism. Dysfunction of chloride transport is found in people with cystic fibrosis. The genetic disorder results from a mutation in a protein called the cystic fibrosis transmembrane conductance regulator [13]. Defects in the protein result in the production of extremely thick mucus that obstructs many of the body's glands and causes many organs, especially the lungs and pancreas, to malfunction.

FUNCTIONS

Chloride has important functions in addition to its role as a major electrolyte. The formation of gastric hydrochloric acid requires chloride, which is secreted along with protons from the parietal cells of the stomach. Chloride is released by white blood cells during phagocytosis to assist in the destruction of foreign substances. Also, chloride acts as the exchange anion for HCO_3^- in red blood cells. This process, sometimes called the chloride shift, requires a protein transporter that moves Cl^- and HCO_3^- in opposite directions across the cell membrane. The purpose is to allow the transport of tissue-derived CO_2 back to the lungs in the form of plasma HCO_3^- . Waste CO_2 from tissues enters the red blood cell, where it is converted to HCO_3^- by carbonic anhydrase. The transporter protein (chloride bicarbonate exchanger) then transports the HCO_3^- out of the cell into the plasma as it simultaneously transports plasma Cl^- into the cell. In the absence of chloride, bicarbonate transport ceases.



EXCRETION

Chloride excretion occurs through three primary routes: the gastrointestinal tract, the skin, and the kidneys, with losses through each route closely reflecting those of sodium. Excretion of chloride through the gastrointestinal tract normally is very small, ~1 to 2 mEq/day for the average adult, and mostly represents unabsorbed chloride. Losses through the skin are essentially the same as for sodium, that is, normally quite small except in cases of high temperature and vigorous exercise. The major route of chloride excretion is through the kidney, where it is primarily regulated indirectly through sodium regulation.

DEFICIENCY

Dietary deficiency of chloride does not occur under normal conditions. As is the case for the other electrolytes, deficiency arises chiefly through gastrointestinal tract disturbance such as severe diarrhea and vomiting. Convulsions typically occur with chloride deficiency.

ADEQUATE INTAKE AND ASSESSMENT OF NUTRITION

The National Research Council recommends an adequate intake amount for chloride of 2,300 mg (65 mmol) per day; this amount is equivalent to sodium on a molar basis [7]. A tolerable upper intake level for chloride is 3.6 g (100 mmol), also equivalent to sodium on a molar basis [7].

The usual serum concentration of chloride is ~101 to 111 mEq/L. Its measurement is generally used to establish the chloride status of the body. However, like all serum solutes, concentration depends on the body water status. It is possible, for example, for total body store of chloride to be diminished and fluid concentrations of chloride to appear normal and even be elevated if body water accompanies the losses. Two widely used methods for determining chloride

concentration in serum are ion-selective electrode potentiometry and a **coulometric titration** (a method of measuring the volume of reagent required for a reaction) with silver ions.

References Cited for Sodium, Potassium, and Chloride

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Macrominerals and Hypertension

Dietary factors influence blood pressure just as they do other physiological processes of the body. Although high blood pressure, called hypertension, has primarily and most publicly been linked to sodium intake, other nutrients play a role in blood pressure control.

Hypertension is thought to affect about 25% of (or ~50 million) Americans. The condition is one in which an increase in vascular resistance occurs, most often caused by decreased luminal diameter of the arteries, arterioles, or both. Systolic and diastolic blood pressure values ≥ 140 and 90 mm Hg, respectively, indicate hypertension. Hypertension is often classified as primary (also called essential) or secondary. Causes of essential hypertension are generally unknown and are thought to be multifactorial, perhaps related to malfunction of sodium excretion or of the renin-angiotensin or kallikrein-kinin systems, hyperactivity of the nervous system, and abnormal prostaglandin production, among other factors. Essential hypertension accounts for <90% of hypertension cases. The remaining cases of hypertension occur secondary to other conditions, such as kidney, endocrine, or neurological diseases. Whether hypertension is essential or secondary, the condition increases the risk of stroke and heart disease. Although some risk factors for hypertension are not controllable (e.g., genetic predisposition, race, aging), others can be modified by a person's commitment to dietary and lifestyle changes. But, genetics matters too, and often influences response. For example, those with an A for G base pair substitution in position six in the promoter region of the angiotensinogen gene had higher angiotensinogen levels. (Higher angiotensinogen levels usually lead to higher blood pressure). Further, when people with the GG genotype (normal) and the AA genotype (variant) were put on a reduced-sodium diet, blood pressure reductions were significantly greater in the AA group than in the GG group [1].

Because hypertension is a heterogeneous disease with a variety of precipitating factors, dietary modification works for some but not all hypertensive people. This Perspective discusses some of the nutrients associated with essential hypertension. The nutrients most often associated with blood pressure are the macrominerals sodium, potassium, calcium, and magnesium. Each of these is discussed in this section, along with sucrose and alcohol, which also influence blood pressure.

Sodium

Sodium, as salt, was one of the first nutrients linked to hypertension. Dozens of studies (epidemiology and observational) have been conducted over several decades examining salt and blood pressure across and within population groups. Study designs and subjects included and excluded have varied as has the means used to assess sodium intake (calculation based on diet rather than on urinary sodium excretion) leading often to conflicting results—no relationship, or a positive relationship between sodium and blood pressure. The Intersalt study, for example, which involved more than 10,000 adults from 32 countries, found that increases in sodium intake of 100 mmol (2,300 mg) was associated with small changes in blood pressure, about 1 to 3 mm Hg increase in systolic and 0 to 2 mm Hg increase in diastolic blood pressure; however, reanalysis of some of the data from this study has found no relationship [2,3]. Intervention studies also have generated conflicting results, or findings that are limited to only some population subgroups [4–9]. Yet, overall, evidence shows relatively consistently that reductions in sodium intake are associated with small reductions in blood pressure.

Blood pressure response to diet is heterogeneous. Some people with hypertension, especially those with a genetic predisposition, appear to be more sensitive to an excess of salt than others. And, in some (about 30% to 50%) but not all hypertensive people, high dietary salt intake raises blood pressure, and dietary salt restriction results in blood pressure reduction. These effects, however, can be modulated by ingesting other nutrients, like potassium (discussed in the next section) [10]. Hypertensive people likely to benefit from sodium reduction include those who are African American, obese, over 65 years of age, those who have low plasma renin activity, those with polymorphisms in genes for angiotensin, as well as those taking antihypertensive medications.

No one mechanism is thought to be responsible for salt-induced elevations in blood pressure. In some people, salt ingestion is thought to cause sodium and water retention and extracellular volume expansion, with the resulting release of a substance or substances that increase heart and blood vessel contractile activity and affect the renin-angiotensin-aldosterone system [11,12]. Alternatively, sodium may infiltrate or cause abnormal handling of other ions into and out of vascular smooth muscle, causing contraction and elevations in blood pressure [11,13]. Sensitivity to sodium correlates with low plasma renin activity (reflecting volume overload), a decreased capacity of the renin-angiotensin

system to respond to physiological stimuli, and insulin resistance [14]. Further, in such people, sodium restriction appears to increase plasma renin activity and sympathetic nervous system activity [14]. Policy recommendations for sodium intake vary with some quite passionate about general recommendations for the population for reductions in salt intake and others believing such recommendations go beyond available data [9].

Potassium

Potassium is yet another nutrient known to affect blood pressure. Epidemiological studies, as well as national surveys, clearly show an association between higher potassium intake (alone or relative to sodium intake) and a lower prevalence of hypertension or lower blood pressure [9,15–19]. Supplementation trials with potassium have further supported such findings. Several meta-analyses of controlled trials found that potassium supplementation was associated with significant reductions in both systolic and diastolic blood pressure [4,20]. Generally, the blood pressure-lowering effects of potassium supplementation were more apparent in hypertensive people than in normotensive people [20–22]. Furthermore, effects of potassium were greater in people ingesting large amounts of sodium [20].

The mechanisms by which potassium affects blood pressure are multiple. Potassium promotes urinary sodium excretion (**natriuresis**) and thus diminishes body sodium. In fact, increased dietary potassium can blunt the rise in blood pressure associated with sodium loading [10]. Increased potassium intake also is associated with reduced urinary calcium and magnesium excretion; calcium and magnesium, discussed in the next two sections, influence blood pressure [23]. Potassium may induce vascular smooth muscle relaxation and thus reduce peripheral resistance. It can inhibit platelet aggregation and arterial thrombosis and also inhibit proliferation of smooth vascular muscle cells to decrease vascular resistance [14]. Potassium may interact with the kinin system; for example, potassium is known to increase urinary kallikrein and also may affect renin to regulate blood pressure [11].

Calcium

A possible relationship between calcium and the development of hypertension was first recognized with the discovery in the early 1970s that communities characterized by hard water (high calcium content) had a lower death rate from cardiovascular disease [24,25]. Since that time, results from epidemiological studies,

laboratory studies involving animals and humans, and clinical trials have accumulated to support the relationship between calcium and blood pressure. A meta-analysis of over 30 randomized calcium supplementation (median intake, 1 g calcium) studies found that calcium supplementation significantly resulted in small reductions in systolic, but not diastolic, blood pressure [26,27]. Further, a meta-analysis of studies involving pregnant women concluded that consuming calcium during pregnancy reduced the risk of pregnancy-induced hypertension [28].

Some segments of the hypertensive population appear to be more responsive to calcium supplementation than other segments. The difference in response to oral calcium among people with hypertension may be attributable to the heterogeneity of the disease. People who appear to benefit from oral calcium therapy are those who have low calcium intakes (especially <400 mg), low serum ionized calcium concentrations, or elevated serum PTH and vitamin D (calcitriol), and those who have been classified as having low renin activity and as being "salt sensitive" [29–31].

How dietary calcium exhibits an antihypertensive effect in "calcium-sensitive" people with hypertension is uncertain. Calcium has a membrane-stabilizing, vasorelaxing effect on the smooth muscle cells [14,31]. It also affects the central and peripheral sympathetic nervous systems and modifies calcium homeostasis as well as the actions of PTH and calcitriol; calcium may suppress PTH-induced elevation in calcium concentration to in turn reduce vascular tone [31]. Increased intracellular calcium concentrations correlate directly with increased blood pressure and age. Calcium may also exert its effects through interaction with other nutrients. For example, increased calcium intake causes natriuresis. Calcium may correct sodium-induced decreases of ionized serum calcium as would occur with sodium-induced calciuria.

Magnesium

Epidemiological data as well as animal and human studies suggest an inverse relationship between blood pressure and magnesium intake. For example, an analysis of 29 observational studies, which examined the relationship between dietary magnesium intake and blood pressure, found a negative association (increased magnesium intake associated with lower blood pressure) [32]. Another meta-analysis of magnesium supplementation trials found dose-dependent reductions in blood pressure with magnesium supplementation [33]. Yet, not all studies

report significant associations between magnesium and blood pressure, or benefits in blood pressure response to magnesium supplementation.

How magnesium directly affects blood pressure is not clear. Magnesium is known to promote relaxation of vascular smooth muscle as well as to interact with calcium [31]. In fact, low serum magnesium (indicative of low magnesium status) is associated with increased smooth muscle tension, vasospasms, and higher blood pressure [12]. Increased blood pressure also is associated with both calcium and magnesium excretion [34]. Magnesium is a required cofactor for enzymes involved in fatty acid metabolism for the synthesis of prostaglandins, and prostaglandins in turn can influence blood pressure. The blood pressure-lowering effects of fish oil, rich in eicosapentaenoic acid and docosahexaenoic acid, have been attributed, in part, to enhanced production of prostaglandins that promotes vasodilation and inhibits platelet aggregation, and to decreased formation of thromboxane A_2 , which promotes vasoconstriction and platelet aggregation.

Other Dietary Factors

Sucrose also has been shown to elevate blood pressure. Several animal studies have demonstrated sucrose-induced rises in blood pressure. The effects are thought to be short term and to result from volume expansion and antinatriuretic effects that accompany sucrose ingestion [22].

Alcohol consumption (especially ingesting three or more drinks per day) is thought to account for up to ~20% of hypertension, especially in middle-aged men [35]. Stimulation of the sympathetic nervous system, changes in hormones (such as renin, angiotensin, aldosterone, insulin, and cortisol), changes in vascular tone (e.g., inhibition of vascular relaxing substances, such as nitric oxide, or increased intracellular concentrations of calcium or electrolytes in vascular smooth muscle), as well as changes in baroreflex sensitivity have been suggested as mechanisms by which alcohol may influence blood pressure [13,35].

The randomized trial Dietary Approaches to Stop Hypertension (DASH) reported that diets rich in fruits, vegetables, and low-fat dairy products but low in fat were more effective in reducing blood pressure than a control diet low in fruits and vegetables and average in fat (~36% of kcal) [34]. The DASH diet provides 3 g sodium, about 4,500 mg potassium, 8 to 10 servings of fruits and vegetables, and 2 to 3 servings of low-fat dairy products; limits red meat, fats, and sugar-sweetened foods and beverages; and emphasizes nuts, seeds, and

legumes. The DASH-sodium study, which compared the effects of the DASH diet with three levels of sodium intake (3.3 g, 2.4 g, and 1.5 g) with those of a control diet, further showed that additional blood pressure reduction could be achieved through reduction in dietary sodium. Similarly, consumption of diets that meet or exceed the recommendations for calcium, potassium, and magnesium is not associated with hypertension, even when the diet is high in sodium chloride [36,37]. Thus, using supplements may not be necessary to achieve reductions in blood pressure. Diets that ensure adequate dietary intakes of calcium, potassium, and magnesium are recommended by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure for the treatment of hypertension [38].

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Osteoporosis and Diet

One in every 3 women and 1 in every 12 men suffers a fracture because of osteoporosis sometime during their lives [1]. Treating the fracture, however, does not necessarily restore health. About 20% of people with hip fractures attributable to osteoporosis die within 1 year of sustaining the fracture [2]. About 33% of people who have had an osteoporosis-induced hip fracture are no longer able to care for themselves and move into nursing homes within the year following the fracture, and another 17% (although they do not require nursing home care) are not able to return to their previous, prefracture lifestyle [3,4].

In the United States, osteoporosis affects approximately 30 to 50 million people, 80% of whom are women. The condition results in about 1.5 million fractures per year. Fractures affecting the spine occur most frequently (over 700,000), followed by fractures of the hip (600,000) and wrist (250,000) [5]. The cost of treating osteoporotic fractures exceeds \$14 billion per year. The condition is considered a major health threat [3].

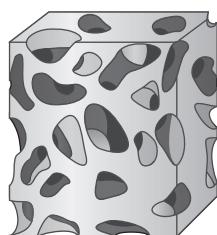
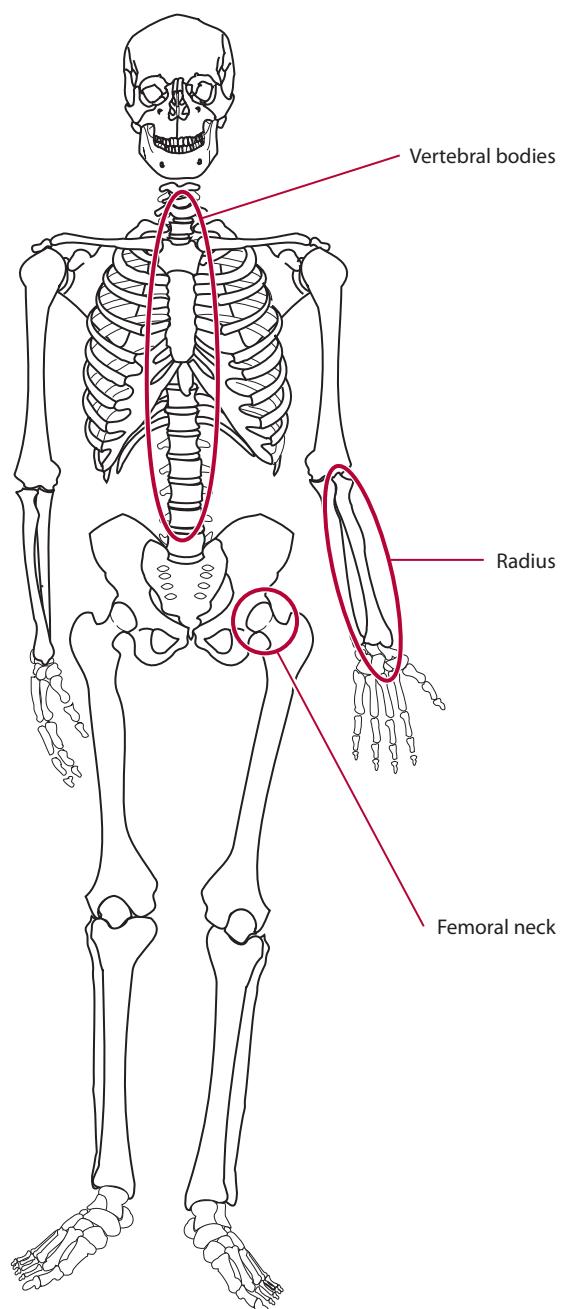
Osteoporosis is a systemic skeletal disease characterized by the deterioration of the microarchitecture of bone tissue and low bone mineral density as shown in Figure 1 [3]. The condition results in fragile bones at increased risk for fracture. Although bone turnover occurs throughout life, after about age 30 to 35 years bone resorption (breakdown) exceeds bone formation. This bone resorption, including mineral loss, occurs at a rate of up to ~10% per decade in both men and women. However, during the first 5 to 8 years after menopause, the rate of loss accelerates considerably in women. The decline in estrogen production that occurs with menopause, coupled with the generally smaller body and bone mass of women, contributes to the higher prevalence of osteoporosis in women than in men.

Osteoporosis affects both cortical and trabecular bone, although trabecular bone has a higher turnover rate and is affected to a greater extent than is cortical

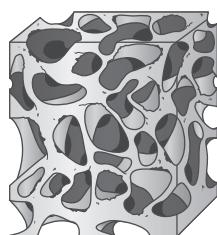
bone. Cortical or compact bone is found mostly in the shaft of long bones of the limbs but also on the outer walls of all bones. Trabecular (or cancellous) bone is the honeycomb or lattice-type bone found in the vertebrae of the spine, the pelvis (hip area), and the ends of long bones. Thus, sites containing trabecular bone—the vertebral bodies (~95% trabecular bone), the femoral neck in the pelvis (~45% trabecular bone), and the radius (~5% trabecular bone)—are the principal sites affected with osteoporosis, especially in women (Figure 2). In addition, teeth also may become loose or fall out because some trabecular bone in the jaw is lost. Osteoporosis that affects the vertebrae is associated with loss in height, vertebral pain, and rounding of the shoulders (**kyphosis**, a hunchback-type curvature of the spine, also called **dowager's hump**). The kyphosis in turn reduces the space in the chest and abdominal cavity, resulting in decreased lung capacity and thus shortness of breath, abdominal pain, reduced appetite, and premature satiety [6]. Many people who suffer an osteoporosis-induced vertebral fracture spend their remaining years in severe, chronic pain and with limited mobility. The limited mobility causes excessive bed rest, which further weakens the person's muscles and bones and predisposes the person to falling and suffering further fractures. The excessive bed rest also increases the person's risk for pressure sores, also called decubitus ulcers.

Two main types of osteoporosis have been described. Primary

osteoporosis is characterized by demineralization of mostly trabecular bone and occurs mostly in postmenopausal women about 50 to 65 years of age, or about 10 to 15 years after menopause. Primary, also called type I osteoporosis or postmenopausal osteoporosis, is linked to menopause and reduced estrogen production. Age-related, also called type II osteoporosis, is characterized by demineralization of both cortical



Normal bone



Osteoporotic bone

Figure 1 Normal bone/osteoporotic bone.

Figure 2 Major sites affected by osteoporosis.

and trabecular bone. It occurs in both men and women over approximately 70 to 75 years of age, although it is more common in women. In type II osteoporosis, trabecular and cortical bone are slowly lost to age-induced decreases in bone cell activity, especially osteoblast activity. In addition, decreased synthesis of calcitriol (caused by decreased 1-hydroxylase activity in the kidney) and decreased intestinal calcium absorption occur with aging and contribute to type II osteoporosis. When these events are coupled with low calcium intake, parathyroid hormone concentrations increase. However, aging in itself also causes mild above-normal elevations in serum parathyroid hormone concentrations. High blood parathyroid hormone concentrations stimulate bone resorption and promote bone demineralization.

Diagnosis of osteoporosis is based on measurements of bone mineral density, primarily by dual-energy X-ray absorptiometry (DEXA). DEXA scans use X-rays at two energy levels to assess bone mineral content. Both peripheral and central DEXA scans are available. The peripheral scan typically measures extremities including the heel, wrist, or finger. Central scans focus on the spine and hip. Software used with the DEXA scan can calculate bone mineral density of various regions of interest. Bone mineral density represents the average concentration of minerals per unit area, or how tightly the bone mineral tissue is compressed in a given area. Bone density is reported for comparison purposes as a T score or Z score. T scores represent the number of standard deviations (SDs) away from the mean bone density of gender- and race-matched young (25- to 45-year-old) adults. A T score equal to 0 means that the person's bone mineral density is at the mean for young adults (considered to represent peak bone mass) of the same gender and race. The World Health Organization [3] defines osteoporosis based on T scores and the number of standard deviations below the young adult mean as shown below:

T Score	Diagnosis
0 to -0.99	Normal
-1 to -2.49	Osteopenia
≤ -2.5	Osteoporosis

For every 1 SD below the mean, the risk of fracture doubles. Thus, those with osteoporosis are at considerable risk for fracture, and those with osteopenia are at risk for fracture as well as for the development of osteoporosis. Z scores are similar to T scores but represent the number of SDs from the mean bone density of age-matched as well as gender- and

race-matched people. Thus, for older people, T scores will likely be lower than Z scores.

The high prevalence of osteoporosis among women makes this condition a public health problem. Women who are most at risk are those with a family history of the disorder, those who are Caucasian or Asian, and those with a small frame size or low body mass index (especially <19 kg/m²). People using medications such as glucocorticoids, thyroid hormones (excess), and antiepileptic drugs also are at increased risk for bone loss and thus osteoporosis. Although the effects of aging and genetic factors (and sometimes drug therapies) cannot be eliminated, other factors contributing to the development of osteoporosis may be modified. Factors interfering with the attainment of peak bone mass as well as factors accelerating the rate of bone loss are influential in the development of osteoporosis. Some of the factors that influence attainment of peak bone mass or accelerate the rate of bone loss are addressed in this Perspective. These factors include estrogen, physical activity, and intakes of calcium, vitamin D, and sodium. In addition, some of the effects of phosphorus, protein, acid-load, vitamins C and K, and fluoride intakes are reviewed, along with the effects of caffeine and alcohol consumption and smoking.

Estrogen

Estrogen has positive effects on bone formation and mineralization, and its influence is especially evident at puberty. Estrogen is produced mainly by the ovaries. Estrogen deficiency promotes bone resorption in all age groups, but in adolescence estrogen deficiency also prevents attainment of peak bone mass. Although estrogen's effects are thought to be mediated by changes in the activities of osteoblasts (bone-forming cells) and osteoclasts (bone-destroying cells), the exact mechanisms by which estrogen affects bone formation and resorption have not been elucidated.

Estrogen concentrations decrease in women around the time of menopause (perimenopause). Estrogen concentrations also are low in women who undergo a surgical ovariectomy (removal of the ovaries), and may be low in young women athletes and in young women with eating disorders, especially anorexia nervosa. This estrogen deficit, whether it occurs in older women at menopause or with an ovariectomy, in adolescent girls, or in young women, increases the risk for development of osteoporosis unless estrogen levels can be quickly restored.

Because of the protective effect of estrogen on bone, many physicians believe that estrogen replacement should be recommended on an individual basis, especially

for women who are immediately postmenopausal or are going through menopause or who have had an ovariectomy. Estrogen replacement in the form of oral contraceptive agents also is important for young women who have low estrogen concentrations because of an eating disorder or considerable physical activity. Moreover, use of oral contraceptive agents in women over the age of 40 years decreases subsequent risk of hip fractures after menopause and improves bone mineral density [7,8]. Estrogen or estrogen replacement therapy (ERT) for peri- or postmenopausal women attenuates bone loss to slow turnover rates and decreases vertebral and nonvertebral fracture rates. It also may increase vertebral and hip bone density [9]. ERT and recovery from amenorrhea, however, have not been associated with normalization of bone density [10]. Moreover, ERT does not protect very well against the spinal dowager's hump, suggesting that bone loss from the spine is likely caused by factors other than (or in addition to) decreased estrogen levels [11].

Use of ERT, however, is not without risk. Major side effects include vaginal bleeding, increased risk of breast cancer as well as of uterine cancer, and increased risk of cardiovascular events [9]. Additional studies are under way to determine whether changing dosage or alternating use of progestins with estrogen might minimize some of the side effects.

Physical Activity

The effects of the absence of physical activity on bone are apparent in people on complete bed rest, because of injury, for example. Similarly, the negative influence of weightlessness, as occurs with space travel, on mineral balance has long been recognized. It follows, therefore, that weight bearing by the bone influences mineral balance positively. This supposition has proved to be true. Weight-bearing exercises, including carrying one's own body weight by walking, running, dancing, or weight training (among other activities), on a regular basis have a protective effect on bone, improving bone mineral density or decreasing the age-related demineralization of bone. Physical activity in college-age women has been positively correlated with rate of gain in spinal bone density [12]. Beyond the benefits to bone, improvements in muscle strength and balance associated with exercise (even walking 4 hours per week) diminish the likelihood of falling and thus of fracturing bones [9,13]. Extreme physical activity, however, when associated with amenorrhea (lack of menstruation and thus low blood estrogen), is counterproductive to maintaining bone mass.

Calcium

Adequate intake of calcium is important throughout life. Sufficient calcium during childhood, adolescence, and young adulthood is especially critical for attainment of the full genetic expression of peak skeletal mass that occurs sometime in early adulthood. Attainment of dense bones during the early years offers the best protection against weakened, osteoporotic bones in later years.

Adequate calcium intake (whether obtained through dietary sources or through food plus supplements) in children and adolescents improves bone mass and bone mineral density and thus help achieve peak bone mass [14,15]. Calcium intake among teenagers, for example, have been shown to correlate with adult bone density [16]. Recommended calcium intakes for children and adults are provided on the inside covers of this book; unfortunately, average calcium intake among females typically is below the recommendations, not only during adolescence but throughout life. The inadequate calcium intake means that less calcium is available to be absorbed and to maintain plasma calcium concentrations. Parathyroid hormone concentrations in turn rise to maintain plasma calcium concentrations, promoting bone resorption. Thus, to prevent osteoporosis, ingesting adequate calcium as well as vitamin D is imperative.

Although peak bone mass is achieved by early adulthood, calcium is needed throughout life for function in bone as well as in other body tissues, such as blood and muscles. The accelerated loss of bone that occurs in women after menopause and the age-related increases in parathyroid hormone concentrations can be ameliorated in part by adequate intake of calcium (typically 1,000–1,200 mg or more) and vitamin D (typically 400–800 IU or more) [3,17–20]. Supplementation with calcium and (typically) vitamin D and estrogen improves or prevents the loss of bone mineral density, especially in the spine, and decreases vertebral and nonvertebral fractures in postmenopausal women [21,22].

Vitamin D

As you may remember from Chapter 10, calcitriol, $1,25-(OH)_2D_3$, stimulates the absorption of calcium from the gastrointestinal tract. Specifically, the vitamin/hormone interacts with receptors in the enterocyte and, following transport to the nucleus, increases transcription of genes that code for calbindin. Calbindin functions as a calcium-binding protein and enhances calcium absorption. Calcitriol is also thought

to induce changes in the intestinal membranes to enhance calcium absorption. Calcitriol also may be involved in the PTH-mediated calcium reabsorption in the kidney and the PTH-mediated calcium resorption by bone.

Although everyone needs to ingest adequate amounts of vitamin D, elderly people appear to benefit from vitamin D supplementation. Poor vitamin D status is common in the elderly because of marginal intake of the vitamin, little exposure to sunlight, and decreased efficiency of transformation of the vitamin into its active metabolite, calcitriol (1,25-dihydroxycholecalciferol) due to decreased renal 1-hydroxylase activity. Furthermore, the amount of vitamin D_3 produced in aging skin during exposure to the ultraviolet rays of the sun may be decreased to half that produced in young skin [23]. Serum 25-hydroxyvitamin D concentrations are used to assess vitamin D status. When concentrations are suboptimal, vitamin D supplements of typically 400 to 1,000 IU effectively improve vitamin D status. In addition, various analogues of vitamin D are being studied for their effectiveness in improving bone formation [24].

Several studies have shown that supplementing both vitamin D and calcium improves bone density and diminishes fractures. For example, vitamin D supplements (400 IU) coupled with calcium supplements (377 mg/day as calcium citrate malate) increased bone mineral density in the spine and decreased risk of vertebral fractures in postmenopausal women [25]. In a study that supplied 1.2 g calcium as tricalcium phosphate and 800 IU vitamin D, the risk of hip and other nonvertebral fractures significantly decreased and proximal femur bone density increased in elderly women [17]. Optimal effective doses of vitamin D associated with osteoporosis treatment range from about 400 to 1,000 IU, although higher doses may be needed [3].

Sodium

Whereas vitamin D improves the body's calcium status, high sodium intake can be detrimental to body calcium. Sodium is excreted in the urine with calcium, so a direct relationship exists between the two nutrients. Moreover, because dietary sodium intake in the United States is much higher than sodium needs, most ingested sodium is excreted in the urine. A sodium load of 100 mmol (2.3 g) per day increases urinary calcium excretion by 0.5 to 1.5 mmol (20–60 mg) per day [26–28]. Thus, if the amount of calcium absorbed is not adequate to compensate quantitatively for the increased urinary

calcium loss, then bone mass may be compromised [28]. Urinary sodium excretion has been negatively correlated with changes in bone density (bone loss) in the hip region of postmenopausal women in one study [29]. However, other studies and analyses have found that high dietary sodium intake does not significantly affect biomarkers of bone resorption or formation, especially in young adults, and that adequate potassium intake can reduce or prevent salt-induced increases in urinary calcium excretion [28,30–32]. Clearly, additional studies are needed.

Phosphorus

Diets adequate in phosphorus are important for bone health. Definitive answers as to the effects of high dietary phosphorus when consumed with low calcium (common in the United States) on bone turnover still cannot be given without further research. What is known is that high plasma phosphorus concentrations, by stimulating parathyroid hormone secretion, increase indirectly the reabsorption of calcium by the renal tubules so that less calcium is lost in the urine. Diets high in phosphorus also are usually high in protein, which may enhance calcium absorption. Yet, phosphorus also causes loss of calcium by increasing calcium secretion into the gastrointestinal tract.

Prolonged ingestion of diets high in phosphorus and low in calcium can result in a mild secondary hyperparathyroidism [33,34]. Thus, increased parathyroid hormone concentrations stimulate bone resorption, with possible long-term detrimental effects on bone mineral content [33–35]. The synthesis of vitamin D (calcitriol, or 1,25-dihydroxycholecalciferol) in response to the elevated parathyroid hormone concentrations varies. Long-term intake of high-phosphorus/low-calcium diets appears to be associated with no rise in calcitriol synthesis, which is needed to improve calcium absorption [33]. However, high-phosphorus diets, although they increase parathyroid hormone concentrations, have not been shown to consistently enhance concentrations of compounds (biomarkers) that indicate increased bone resorption or turnover [33,36–38]. Moreover, bone resorption has been found to be lower when plasma phosphorus concentrations are higher (versus lower) at any given parathyroid hormone concentration [33,35]. Clearly, further research to address the effects of high-phosphorus diets (alone and coupled with varying levels of dietary calcium) on both bone resorption and bone accretion is needed to clarify the effects of diets high in phosphorus on the development of osteoporosis.

Protein

Adequate protein intake is necessary for bone health, yet concerns have been raised that high protein intake (especially from animal sources) may be detrimental to bone (as a risk factor for osteoporosis). The high-protein diet is thought to be associated with increased dietary sulfur intake (as sulfur-containing amino acids) and theoretically cause calcium to be “pulled” out of bone to neutralize the excess acid load resulting from the high-protein (sulfur) diet (see also the next section on acid load). Dietary protein directly influences calcium; doubling protein intake without changing intake of other nutrients results in about a 50% increase in urinary calcium [39–42]; however, the rise in urinary calcium is associated with increased calcium absorption and is not associated with increased bone resorption [43–45]. Moreover, in natural foods, proteins are usually combined with substances that counteract protein’s effect on calcium excretion [46]. Large prospective epidemiological observations together with intervention studies suggest that diets relatively high in protein are associated with increased bone mineral mass and reduced incidence of osteoporotic bone fractures [43]. In a group of college women, the rate of gain of spinal bone density was positively correlated with the calcium:protein intake ratio [12]. Moreover, in recovery from bone fractures, higher intakes of both energy and protein have been shown to improve recovery times and to attenuate the decrease in bone mineral density associated with the fracture [47,48]. Inadequate protein intake negatively affects bone health and healing from fractures. Further, protein supplementation or a high-protein diet appears to enhance production of bone growth factor (IGF-1), which promotes skeletal development and bone formation [43,45].

Acid Load

Acid ash is produced in the body in varying amounts based on the foods consumed; this ash in turn must be eliminated or handled effectively in the body to prevent problems such as metabolic acidosis and its possible damage. Ingesting meat, fish, eggs, cheese (and to a lesser extent, most grain products), for example, generates acid ash in the body. Most of the acids generated from these foods are thought to arise from oxidation of the sulfur-containing amino acids, which produces sulfur-containing acids in the body. Consuming soft drinks (among other foods like citrus products) also provides considerable amounts of acids (especially phosphoric acid with soft drink consumption)

that are absorbed into the body. Excess acids in the body are buffered in the blood by various compounds and are excreted by the kidneys in the urine; however, the pH of the urine can only go so low—usually not less than 5. Some suggest that a low-grade metabolic acidosis (in the blood) is generated by ingesting large amounts of protein-rich foods and soft drinks, and by ingesting inadequate amounts of fresh fruits and vegetables rich in potassium and anions like citrate, which form bicarbonate (to buffer the acid) in the body. Citrate or alkaline salts, like potassium citrate or potassium bicarbonate, are thought to be important to neutralize endogenous acids produced in the body. If the kidneys are unable to excrete the excess hydrogen ions and if available buffers (like bicarbonate) are insufficient, a low-grade acidosis may result. To buffer the blood, the hydrogen ions are thought by some (but not others) to be exchanged with carbonate and minerals, such as calcium, sodium, and potassium, from bone. Thus, the acidosis is corrected, but at the expense of bone minerals [49,50]. Others contend that kidneys and other buffers in the blood correct any imbalances (assuming kidney function is normal and the actions of other buffers are normal) and that bones, which are not in direct contact with systemic circulation, are not affected by diets high in protein or acids, as long as the diet is adequate in other food groups [43,45,51]. Studies providing potassium bicarbonate in place of potassium chloride report significant reductions in urinary calcium excretion and in markers of bone resorption [52]. Other studies also have found that potassium bicarbonate supplementation (60–120 mmol/day) improved calcium balance, reduced bone resorption, and increased the rate of bone formation [53]. More studies appear to be needed to better determine to what extent acidosis occurs and if it poses a true risk for osteoporosis.

Vitamins C and K

Vitamins C and K are important for the synthesis and function of various proteins found in bone. Collagen is one of the main proteins found in bone, and the synthesis of collagen is dependent on vitamin C. Positive correlations between vitamin C intake and bone mineral density have been shown in adolescents and in adult women [54,55].

In addition to collagen, bone also contains many other proteins, including osteocalcin and matrix Gla protein. Osteocalcin and matrix Gla protein require vitamin K to function. With inadequate vitamin K status, these two proteins are not carboxylated as they normally would be and thus have limited ability to bind calcium and

aid in bone mineralization. Serum undercarboxylated osteocalcin concentrations (a sign of poor vitamin K status) have been found to be correlated with bone mineral density in the **Ward’s triangle** (a region within the hip) and femoral neck in women during the first decade of menopause [56]. Serum undercarboxylated osteocalcin concentrations also have been shown to predict increased risk of hip fractures in elderly women [57]. In addition, low vitamin K intake has been found to be associated with an increased incidence of hip fractures in elderly men and women [58].

Fluoride

Use of fluoride is not recommended or approved for the prevention or treatment of osteoporosis [4,59]. Although fluoride reduces the incidence of dental caries, its effectiveness in preventing and treating osteoporosis is inconsistent. Fluoride, usually administered as sodium fluoride (40–80 mg/day), stimulates bone formation (osteoblast activity). In some studies, use of fluoride along with calcium increased mostly trabecular bone mass and to some extent cortical bone and decreased fracture rates in postmenopausal women [60–62]. However, supplements of 75 mg fluoride together with 1,500 mg calcium failed to reduce the risk of vertebral fractures and increased the risk of nonvertebral fractures in postmenopausal women [63]. In addition, abnormal bone quality has been found to accompany increases in bone density following use of fluoride. Other research has shown that highly fluoridated water fails to protect against bone loss [64]. Although the formulation, dose, delivery mode, and duration of fluoride therapy may account for observed differences among studies, additional studies are needed before recommendations for fluoride therapy may be issued [60,61,63].

Smoking

Smoking negatively affects bone health. Smoking is associated with lower bone density and, in women, with earlier menopause and increased postmenopausal bone loss [65,66]. Smoking decreases circulating estrogen concentrations, thereby contributing to bone loss [65]. Smoking has also been shown to be a significant predictor of bone loss in men [67] and is associated with increased risk of fractures at various sites in men and women.

Alcohol

Chronic and excessive ingestion of alcohol damages bone and increases the risk of osteoporosis. The mechanisms by which alcohol exerts its effects are unclear but are thought to be multifactorial [68].

Alcohol consumption has been significantly associated with increased rates of bone loss in men [67]. People consuming excessive alcohol generally have lower bone mass and reduced osteoblast activity and are at increased risk, in a dose-response relationship, of hip and forearm fractures [68,69]. Factors associated with excessive alcohol intake affecting bone loss include insufficient intake of nutrients (especially calcium, protein, and vitamin D) coupled with poor absorption of nutrients, as well as elevated parathyroid hormone concentrations [68].

Caffeine

Caffeine minimally affects calcium balance and therefore is thought to be weakly associated with the development of osteoporosis. Caffeine reduces the renal reabsorption of calcium, which leads to a temporary (about 1 to 3 hour) increase in urinary calcium losses. The loss is typically followed by a period of reduced urinary calcium excretion with no net effect [51,70]. It has been estimated that 1 cup of caffeinated coffee promotes the loss of only about 6 mg calcium in the urine [69,71]. Caffeine in amounts of 300 to 400 mg increased urinary calcium by 10 mg/day [72]. However, caffeine may also promote increased secretion of calcium into the gut to enhance calcium loss from the body; whether the secreted calcium is reabsorbed, and the extent of the secretion, have not been determined. Caffeine intake has been positively associated with risk of hip fracture in middle-aged women, especially those whose calcium intake is low [69]. However, no association was reported between current caffeine intake and bone density in postmenopausal women [73].

Other Factors

Maintenance of desirable skeletal status clearly is multifactorial. Although nutrients such as calcium and vitamin D play considerable roles in bone health, many other nutrients (some probably still undiscovered) play minor but important roles. Inadequate amounts of dietary boron or magnesium, for example, can promote bone problems. Moreover, diets with added copper, manganese, and zinc, for example, have been shown to be more effective in arresting bone loss in postmenopausal women than diets with no added nutrients or with only added calcium or trace minerals [74]. On the other hand, excess nutrient intake also can be detrimental. Too much vitamin A (retinol), over about 1.5 mg for example, has been associated with losses in bone mineral density and increased risk of hip fracture in some but not all studies [75–77].

Summary

A person's genetic makeup cannot be changed, nor can the physiological changes accompanying aging be reversed. The person usually does have the option, however, of choosing a lifestyle in which good nutrition (i.e., eating a variety of foods—especially fruits and vegetables—and getting recommended intakes of all nutrients) and weight-bearing exercise are practiced regularly [78]. In addition to a good diet and exercise, attention to the hormonal environment is also critical to attenuating bone loss during periods of low estrogen concentration such as may occur with eating disorders or excessive exercise or during the peri- and postmenopausal stages of life for women. With bone density monitoring and early diagnosis of problems, appropriate interventions may be started to slow or halt the progression of osteoporosis [5]. The National Osteoporosis Foundation recommends drug therapy for women with T scores (based on DEXA determined at the hip) below -2 with no other risk factors for fractures, or for women with a T score below -1.5 who have one or more risk factors for fractures [79]. In addition, anyone with a prior vertebral or hip fracture should receive treatment [79]. See the article by Mayes [80] for a review of drug therapies available for the treatment of osteoporosis.

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Suggested Reading

Bone Health and Osteoporosis. A report of the Surgeon General. Rockville, MD: U.S. Dept of Health and Human Services, 2004.

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Iron
Zinc
Copper
Selenium
Chromium
Iodine
Manganese
Molybdenum

Fluoride

For each of the microminerals listed, the following subtopics (if known and when applicable) are discussed:

- Sources
Digestion, Absorption, Transport, Uptake, and Storage
Functions and Mechanisms of Action
Interactions with Other Nutrients
Excretion
Recommended Dietary Allowance or Adequate Intake
Deficiency
Supplements
Toxicity
Assessment of Nutriture

PERSPECTIVE
Nutrient–Drug Interactions

A precise definition for the essential microminerals (or trace minerals or trace elements) has not been established. These minerals initially gained the description “trace” because their concentrations in tissue were not easily quantified by early analytical methods. Today, however, trace minerals can be analyzed by a variety of techniques. The term *trace* when applied to minerals or elements is still used and can be defined as minerals that make up <0.01% of total body weight [1]. Others define trace elements as nutrients the body needs in concentrations of one part per million or less [1]. Iron appears to be the mineral that divides the macrominerals from the microminerals; consequently, some define an essential trace mineral as a mineral needed by the body in a concentration equal to or lower than that of iron [2]. Alternately, *trace* may be applied to minerals needed by the body in amounts <100 mg per day.

The term *essential* as applied to trace elements also was specified in the 1980s. An element is considered essential if a dietary deficiency of that element consistently results in a suboptimal biological function that is preventable or reversible by physiological amounts of the element [3]. More stringent criteria [4] proposed to establish essentiality of a mineral include the following conditions:

- It is present in all healthy tissue of living things.
- Its concentration from one animal to the next is fairly constant.
- Withdrawing it from the body induces reproducibly the same physiological and structural abnormalities, regardless of species studied.
- Adding it either reduces or prevents these abnormalities.
- The abnormalities induced by deficiencies are always accompanied by specific biochemical changes.
- These biochemical changes can be prevented or cured when the deficiency is prevented or cured.

Elements established as essential may not necessarily comply with all the criteria listed, in part because of limitations imposed by the degree of sophistication of the analytical methodology available. Essentiality, therefore, is technically easier to ascertain for elements that occur in relatively high concentration than for those ultratrace elements occurring at very low concentrations and having a low requirement. Figure 12.1 shows the periodic table and some of the essential trace elements.

For six essential trace minerals (iron, zinc, copper, iodine, selenium, and molybdenum), recommended dietary allowances (RDAs) have been established for humans. Adequate intakes have been estimated for another three trace minerals (fluoride, manganese, and chromium). The inside covers of this book provide the recommended intakes for the microminerals. Very little is known

Figure 12.1 The periodic table highlighting some of the essential trace elements.

about the need for ultratrace elements, including nickel, silicon, vanadium, arsenic, and boron; therefore, no recommendations for intake exist.

Each essential trace mineral is necessary for one or more functions in the body, and its function or functions, like those of other essential nutrients, are optimal when mineral intake and body concentrations of the nutrient fall within a specific range. Whenever the intake or body concentration is too low or too high, function is impaired and death can result.

This chapter describes the sources, digestion, absorption, transport, functions, interactions with other nutrients, excretion, recommended intakes, deficiency, toxicity, and assessment of nutriture for the microminerals. Chapter 13 addresses these topics for several ultratrace elements. Table 12.1 provides an overview of the trace elements, including information on selected functions, approximate body content, sources, deficiency symptoms, and recommended intakes. As noted at the beginning of Chapter 11, the differences in body content between the macro and microminerals is quite large. The body's content of the macrominerals ranges from ~35 to 1,400 g, and that of the trace elements ranges from <1 mg to ~4 g (remember that an ounce weighs about 28.4 g).

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Iron

The human body contains ~2 to 4 g iron, or ~38 mg iron/kg body weight for women and ~50 mg iron/kg body weight for men. Over 65% of body iron is found in hemoglobin, up to about 10% is found as myoglobin, about 1% to 5% is found as part of enzymes, and the remaining body iron is found in the blood or in storage. Table 12.2 gives an approximate distribution of iron per kilogram of body weight in adults [1–3]. The total amount of iron found in a person not only is related to body weight but also is influenced by other physiological conditions, including age, gender, pregnancy, and state of growth.

Iron, a metal, exists in several oxidation states varying from Fe^{6+} to Fe^{2-} , depending on its chemical environment. The only states that are stable in the aqueous environment of the body and in food are the ferric (Fe^{3+}) and the ferrous (Fe^{2+}) forms.

SOURCES

Although iron is widely distributed in food, its content in an average American diet is estimated at 5 to 7 mg iron

Table 12.1 The Microminerals: Approximate Body Content, Selected Function, Deficiency Symptoms, Food Sources, and Recommended Intake Dietary Allowance (RDA) or Adequate Intake (AI)

Mineral	Approximate Body Content	Selected Physiological Roles	Selected Enzyme Cofactor Roles	Selected Deficiency Symptoms	Food Sources	RDA or AI (Adults)
Chromium	4–6 mg	Normal use of blood glucose and function of insulin		Glucose intolerance, glucose and lipid metabolism abnormalities	Mushrooms, prunes, asparagus, organ meats, whole-grain breads and cereals	35 µg* male; 25 µg* female
Copper	50–150 mg	Utilization of iron stores, lipids, collagen, pigment, neurotransmitter synthesis	Oxidases, monooxygenases, superoxide dismutase	Anemia, neutropenia, bone abnormalities	Liver, shellfish, whole grains, legumes, eggs, meat, fish	900 µg
Fluoride	Unknown	Maintenance of teeth and bone structure		Dental caries, bone problems	Fish, meat, legumes, grains, drinking water (variable)	4 mg* male; 3 mg* female
Iodine	15–20 mg	Thyroid hormones synthesis		Enlarged thyroid gland, myxedema, cretinism, increase in blood lipids, gluconeogenesis, and extracellular retention of NaCl and H ₂ O	Iodized salt; salt-water seafood, sunflower seeds, mushrooms, liver, eggs	150 µg
Iron	2.4 g	Component of hemoglobin and myoglobin for O ₂ transport and cellular use	Heme enzymes, catalase, cytochromes, myeloperoxidase, nonheme enzymes, camitine and collagen synthesis	Listlessness, fatigue, anemia, palpitations, sore tongue, angular stomatitis, dysphagia, decreased resistance to infection	Organ meats (liver), meat, molasses, clams, oysters, nuts, legumes, seeds, green leafy vegetables, dried fruits, enriched/whole-grain breads/cereals	8 mg male; 18 mg female
Manganese	10–20 mg	Brain function, collagen, bone, growth, urea synthesis, glucose and lipid metabolism, CNS function	Arginase, pyruvate carboxylase, PEP, carboxykinase, superoxide dismutase	In animals, possibly humans: impaired growth, skeletal abnormalities, impaired CNS function	Wheat bran, legumes, nuts, lettuce, beet tops, blueberries, pineapple, seafood, poultry, meat	2.3 mg* male; 1.8 mg* female
Molybdenum	Unknown	Metabolism of purines, pyrimidines, pteridines, aldehydes, and oxidation	Xanthine dehydrogenase/oxidase, aldehyde oxidase, sulfite oxidase	Hypermethioninemia, ↑ urinary xanthine, sulfate excretion, ↓ urinary sulfate and urate excretion	Soybeans, lentils, buckwheat, oats, rice, bread	45 µg
Selenium	15 mg	Protects cells against destruction by hydrogen peroxide and free radicals	Glutathione peroxidase, 5'-deiodinase, thioredoxin reductase	Myalgia, cardiac myopathy, ↑ cell fragility, pancreatic degeneration	Grains, meat, poultry, fish, dairy products	55 µg
Zinc	1.5–2.5 g	Energy metabolism, protein synthesis, collagen formation, alcohol detoxification, carbon dioxide elimination, sexual maturation, taste and smell functions	DNA-RNA polymerase, carbonic anhydrase, carboxypeptidase, alkaline phosphatase, deoxythymidine kinase	Poor wound healing, subnormal growth, anorexia, abnormal taste/smell; changes in hair, skin, nails; retarded reproductive system development	Oysters, wheat germ, beef, liver, poultry, whole grains	11 mg male; 8 mg female

*Indicates adequate intake.

Table 12.2 Approximate Distribution of Iron in Adult Males and Females (mg/kg body weight)

	Males	Females
Functional iron		
Hemoglobin	31	28
Myoglobin	5	4
Heme enzymes	1	1
Nonheme enzymes	1	2
Transport iron		
Transferrin	0.05	0.05
Storage iron		
Ferritin and hemosiderin	12	4
Total iron:	50.05	39.05

per 1,000 kcal. Dietary iron is found in one of two forms in foods, heme and nonheme. Heme iron represents iron that is contained with the porphyrin ring structure shown in Figure 12.2. Heme iron is derived mainly from hemoglobin and myoglobin and thus is found in animal products, especially meat, fish, and poultry. About 50% to 60% of the iron in meat, fish, and poultry is heme iron; the rest is nonheme iron. Nonheme iron is found primarily in plant foods (nuts, fruits, vegetables, grains, tofu) and dairy products (milk, cheese, eggs), although dairy products have very little iron and represent a very poor iron source. Nonheme iron is usually bound to components of foods and must be hydrolyzed, digested, or solubilized in the gastrointestinal tract before being absorbed into the intestinal cells.

Foods particularly high in iron, such as liver and organ meats, are not popular items in most American diets. More popular foods that are relatively good sources of iron include red meats, oysters and clams, beans (lima, navy), dark green leafy vegetables, and dried fruits. Other good sources of iron are listed in Table 12.1.

In addition to amounts of iron found naturally in foods, foods such as breads, rolls, pasta, cereals, grits, and flour are fortified with iron. Fortified flour, for example, contains 20 mg iron per lb, and corn grits, corn meal, and rice contain from 13 to 26 mg per lb. Pasta has 13 to 16.5 mg per lb, and bread, rolls, and buns contain 12.5 mg iron per lb. Elemental iron, ferrous ascorbate, ferrous carbonate, ferrous citrate, ferrous fumarate, ferrous gluconate, ferrous lactate, ferric ammonium citrate, ferric chloride, ferric citrate, ferric pyrophosphate, and ferric sulfate are approved and used for food fortification.

DIGESTION, ABSORPTION, TRANSPORT, STORAGE, AND UPTAKE

Heme Iron Digestion and Absorption

Heme iron must be hydrolyzed from the globin portion of hemoglobin or myoglobin before absorption. This digestion is accomplished by proteases in both the stomach and the small intestine and results in the release of heme iron from

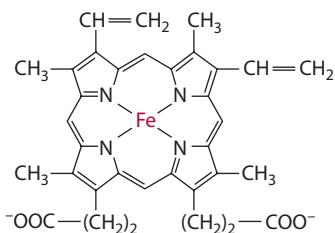


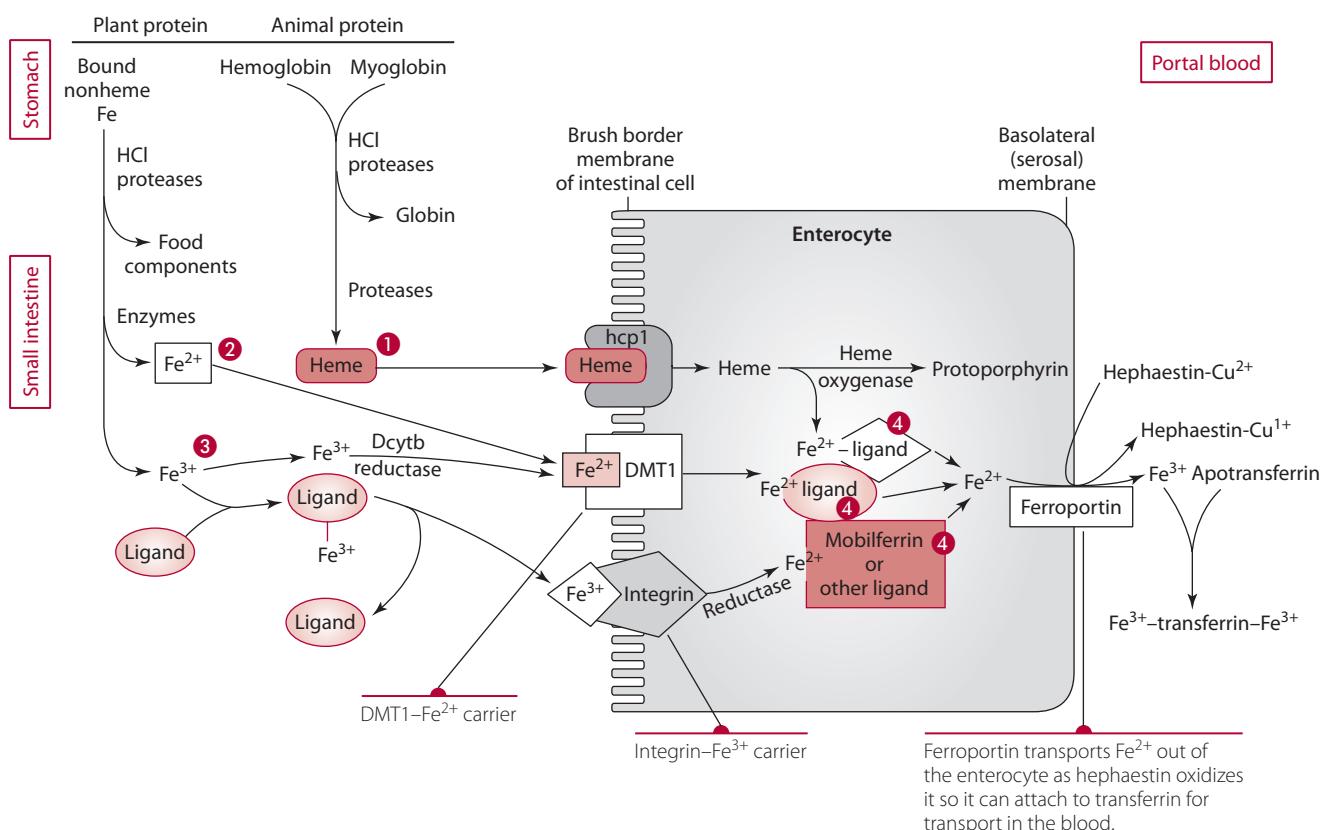
Figure 12.2 Heme iron, a metalloporphyrin.

the globin. Heme, containing the iron bound to the porphyrin ring (also called a metalloporphyrin; Figure 12.2), remains soluble, especially in the presence of the degradation products (amino acids and peptides) of globin, and is readily absorbed intact across the brush border of the mucosal cell (enterocyte) by heme carrier protein 1 (abbreviated hcp1). Heme carrier protein is found mainly in the proximal small intestine. Iron absorption occurs throughout the small intestine, but it is most efficient in the proximal portion, particularly the duodenum. Within the mucosal cell, the absorbed heme porphyrin ring is hydrolyzed by heme oxygenase into inorganic ferrous iron and protoporphyrin (Figure 12.3). The released iron may associate with proteins such as mobilferrin that make up the paraferritin complex (described in the “Nonheme Iron Digestion and Absorption” section) and can be used by the intestinal mucosal cell, excreted with the sloughing of the enterocytes, or, following transport out of the enterocyte, used by other body tissues.

Nonheme Iron Digestion and Absorption

Nonheme iron bound to components of foods must be enzymatically freed (hydrolyzed) in the gastrointestinal tract to be absorbed (Figure 12.3). Gastric secretions, including hydrochloric acid and proteases in the stomach and small intestine, aid in the release of nonheme iron from food components.

Once released from food components, most nonheme iron is present as ferric (Fe^{3+}) iron in the stomach. Ferric iron remains fairly soluble as long as the pH of the environment is acidic. Some of the ferric iron may be reduced to the ferrous state (Fe^{2+}) in the stomach. Once the iron passes from the stomach into the small intestine, ferric iron mixes with alkaline juices secreted into the intestine from the pancreas. In this more alkaline environment, ferric iron may complex to produce ferric hydroxide (Fe(OH)_3), a relatively insoluble compound that tends to aggregate and precipitate, making the iron less available for absorption. In contrast to ferric iron, ferrous iron remains fairly soluble at a more alkaline pH, although some ferrous iron may be oxidized in the alkaline pH of the intestine to the ferric form. Ferrireductases, including ferric/cupric duodenal cytochrome b (Dcytb) reductase, have been identified on the brush border membrane of enterocytes and function in the duodenum to reduce ferric iron to the ferrous state. Vitamin C appears to be needed for reductase activity [4].



- ① Heme is released from hemoglobin and myoglobin and transported into the enterocyte by heme carrier protein (hcp1). In the cell, Fe²⁺ is released from the heme by heme oxygenase.
- ② Fe²⁺, released from food components, is mainly transported into cells by divalent mineral transporter (DMT1).
- ③ Fe³⁺, released from food components, typically binds to ligands (like mucin) to maintain solubility. It is then reduced to Fe²⁺ by reductases or transported (and reduced) by integrin to enter the intestinal cell.
- ④ Within the enterocyte, iron is bound to various ligands including mobilferrin and amino acids for transport to the basolateral membrane.

Figure 12.3 Overview of iron digestion, absorption, and transport.

Thus, following iron's release from food components, nonheme iron may be present in either the ferric or the ferrous state in the small intestine. Ferrous iron may be absorbed across the brush border membrane and into the intestinal mucosal cell by binding to transporters located in the intestinal cell brush border membrane. The main transporter is divalent cation (also called mineral) transporter 1 (abbreviated DCT or DMT); hereafter, the transporter is abbreviated DMT1. In the gastrointestinal tract, the DMT1 transporters are found primarily in the duodenum and transport not only iron but also, to a lesser extent, other minerals such as zinc, manganese, copper, nickel, and lead. Mineral transport using DMT1 is coupled with H⁺ transport (symport) into the enterocyte. Synthesis of DMT1 is affected by iron status, with increased transporter synthesis associated with low iron stores.

The mechanism or mechanisms by which ferric iron is absorbed are not clearly delineated. Absorption from an acidic environment is best and is facilitated by chelation of the iron with ligands or **chelators** (see “Factors Influencing Iron Absorption”) that help solubilize the ferric iron.

A membrane protein called integrin is thought to facilitate ferric iron (and zinc) absorption across the brush border membrane of the enterocyte. Integrin is thought to exist as part of the paraferritin complex, which includes mobilferrin and a flavin-dependent ferrireductase. The roles of mobilferrin and ferrireductase in transporting and reducing iron in the cytosol are covered in the section “Intestinal Cell Iron Use”. The role of ligands and chelators in the absorption of iron is described in the next section.

Factors Influencing Iron Absorption

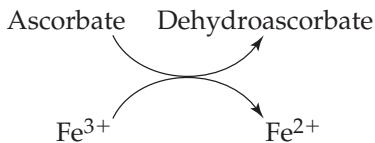
Several compounds (known as chelators or ligands) may bind with nonheme iron to either inhibit or enhance its absorption. Chelators are small organic compounds that form a complex with a metal ion. Ligands are compounds that also bind or complex with minerals. Whether chelated iron or iron attached to a ligand is absorbed or not absorbed depends in part on the nature of the iron-chelate/ligand complex. If the iron-chelate/ligand complex maintains solubility and the iron is loosely bonded, the iron typically can be released at the mucosal cell and absorption enhanced.

However, if the iron chelate/ligand is strongly bonded and insoluble, iron is not absorbed but is excreted in the feces as part of the chelate.

Enhancers of Iron Absorption Some dietary factors that have been found to enhance nonheme iron absorption include:

- sugars, especially fructose and sorbitol
- acids, such as ascorbic, citric, lactic, and tartaric
- meat, poultry, and fish or their digestion products
- mucin

Ascorbic acid (vitamin C), along with citric, lactic, and tartaric acids, for example, acts as a reducing agent and forms a chelate with nonheme ferric iron at an acid pH.



This chelate (a ferrous ascorbate chelate if vitamin C served as the reducing agent) remains soluble in the small intestine and thus can improve intestinal absorption of nonheme iron.

Meat, poultry, and fish factors that enhance nonheme iron absorption have not been clearly identified. Digestion products from animal tissues high in the contractile proteins actin and myosin promote iron absorption [5]. These proteins are digested into peptides that contain relatively large amounts of the amino acid cysteine, which is believed to serve as a ligand to facilitate iron absorption [5]. Another amino acid histidine also may chelate iron to enhance its absorption. Meat is further suspected to improve iron absorption by stimulating intestinal secretions [5].

The amount of iron available for absorption can be estimated from the quantity of vitamin C and meat, fish, or poultry that is ingested with the nonheme iron source, assuming ~500 mg body iron stores. Seventy-five units of ascorbic acid or meat, fish, or poultry (MFP) factor (one unit = 1.3 g raw or 1 g cooked meat, fish, or poultry or 1 mg ascorbic acid) has been shown to maximize iron absorption when consumed with the iron source [6]. Units in excess of 75 seem to have no further benefit. The absence of enhancing factors predicts a nonheme iron absorption of only 2% to 3%, but 75 units of these factors can increase absorption of nonheme iron to 8% (some suggest up to 20% if the person is also iron deficient) [7].

Mucin, an endogenously synthesized chelator, is a small protein made in both gastric and intestinal cells. Gastric mucin (sometimes called gastroferrin) is released into the lumen of the gastrointestinal tract, and some mucin is also found on the brush border membrane of mucosal cells in the intestine. Chelation of iron by mucin facilitates iron

absorption. Mucin binds multiple ferric iron atoms at an acid pH and maintains ferric iron solubility in the alkaline pH of the small intestine. Histidine, ascorbic acid, and fructose, other chelators of iron, are thought to donate the iron to mucin in the small intestine. In addition to iron, mucin also binds and facilitates absorption of zinc and chromium.

Inhibitors of Iron Absorption Many dietary factors inhibit iron absorption, including:

- polyphenols such as tannin derivatives of gallic acid (in tea and coffee)
- oxalic acid (in spinach, chard, berries, chocolate, and tea, among other sources)
- phytates, also called phytic acid, inositol hexaphosphate, or polyphosphate (in maize, whole grains, legumes)
- phosvitin, a protein containing phosphorylated serine residues found in egg yolks
- nutrients such as calcium, calcium phosphate salts, zinc, manganese, and nickel

Polyphenols are found in fairly high concentrations in both tea and coffee. These phenolic compounds, when consumed with a source of iron, can reduce iron absorption over 60%. Coffee consumption, with or just after a meal, may reduce iron absorption by 40% [8].

Phytates and oxalates use oxygen to bind with many minerals, including not only iron but also zinc, copper, and calcium. The phytate mineral and oxalate mineral complexes are insoluble and poorly absorbed. Fermentation of bread reduces the phytate content and improves the absorption of some minerals, but, in general, mineral absorption is better without the presence of phytates or oxalates. (Figure 12.10, in the section on zinc, shows the structures of both phytate and oxalate.)

Several nutrients, when ingested in large amounts, can reduce absorption of nonheme iron. Calcium and phosphorus are thought to interact with iron and inhibit its absorption through Fe:Ca:PO_4 chelate formation at the intestinal mucosa. Alternately, the inhibitory effect of calcium on iron absorption may be within the intestinal mucosal cells at a step in iron transport that is common for both heme and nonheme iron transport [9]. Several studies [9–12] have demonstrated that calcium in amounts of 300 to 600 mg and in the forms of calcium phosphate, calcium citrate, calcium carbonate, and calcium chloride, when given with up to 18 mg iron as ferrous sulfate or when incorporated into food, substantially decreases iron absorption by up to 70%. Similar reductions in iron absorption have been shown with milk ingestion [11]. Thus, those with iron deficiency who need to maximize iron absorption from a supplement should not take the iron supplement with a source of calcium.

Zinc and iron also interact and may negatively affect each other's absorption. The two minerals are thought to

compete for the same transporters, such as DMT1, as well as to interact at another, more distal, site. Inhibition of iron absorption has been demonstrated with the coingestion of zinc, usually as zinc sulfate, in amounts greater than iron as ferrous sulfate. For example, ingesting 15 mg and 45 mg zinc as zinc sulfate, given in a water solution with 3 mg iron as ferrous sulfate, significantly reduced iron absorption [13]. Zinc in a 1:1 and a 2.5:1 (27 mg zinc and 68.5 mg zinc doses) molar ratio with iron in solution inhibited nonheme iron absorption by 66% and 80%, respectively [14]. A review of studies assessing iron and zinc interactions suggests that the interactions result primarily when the two minerals are given in solution and do not occur when they are given in a meal [15]; however, a study in which flour was cofortified with equal amounts of iron and zinc as zinc sulfate significantly reduced iron absorption in children [16].

Manganese and iron also appear to interact. Manganese (as manganese chloride) when ingested in water or with a meal in a 2.5:1 or 5:1 ratio with iron (as ferrous sulfate) reduced iron absorption by 22% to 40% [13].

Other intraluminal factors inhibitory to iron absorption include rapid transit time, malabsorption syndromes, achylia (absence of digestive juices), and excess alkalinization as may occur with excessive use of antacids or with decreased gastric acidity. Overall absorption of iron from the U.S. diet is estimated at about 10% to 18%, but a person's iron status also affects iron absorption.

Iron absorption is closely tied to the level of the body's iron stores. Absorption, for example, may range from about 10% (for a person with normal iron status) up to about 35% (for persons who are iron deficient) [17]. In other words, iron absorption can rise to 3 to 6 mg daily when the body is depleted of iron and can fall to 0.5 mg or less daily when iron stores are high. Additional information on the regulation of iron absorption follows the section about intestinal cell iron use.

Intestinal Cell Iron Use

The preceding sections have reviewed digestion and absorption, including factors inhibiting and enhancing iron absorption into the enterocyte. Following absorption across the brush border membrane into the enterocyte, iron can be handled in one of three ways:

- transported through the enterocyte cytosol and across the basolateral membrane of the intestinal cell to enter circulation for transport to body tissues
- stored in the intestinal cell for future use or elimination
- used by the intestinal cell in a functional capacity

This section first describes transport across the enterocyte.

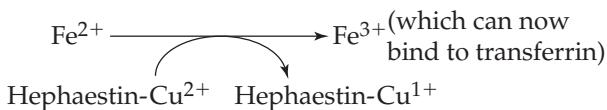
Because of the potential for free iron to initiate oxidative damage, little iron is thought to freely exist within the cytosol of the mucosal cell. Proteins, amino acids, or both are thought to transport or ferry the iron throughout

the cell. Cysteine and histidine are two examples of amino acids thought to be able to transport iron across the mucosal cell. In addition, ferric iron may bind to the cytosolic protein mobilferrin. Two forms of mobilferrin exist: monomeric mobilferrin and mobilferrin that is part of a paraferritin complex. Mobilferrin (both forms) appears to interact with integrin (and possibly DMT1), located in the enterocyte membrane. Mobilferrin binds minerals, especially iron but also calcium, zinc, and copper. Specifically, mobilferrin is capable of binding one iron atom, which it then shuttles across the cytosol of the mucosal cell. Also present with mobilferrin in the paraferritin complex is a NADPH-dependent ferrireductase that reduces ferric iron to its ferrous state (this ferrireductase is called paraferritin in some literature). Flavins (FAD/FADH₂), NAD/NADH, or vitamin C also may reduce the ferric iron within the cytosol. Whether or not other proteins function as **chaperones** (soluble intracellular proteins that bind intracellular components and deliver them to various locations) for iron transport within the cytosol of the cells is not clear.

Iron not being transported across the cell for release into the blood may be incorporated into apoferritin in the intestinal cell for short-term storage. Apoferritin is a protein that acts as a "shell" for iron storage. This protein's shell further serves as a ferroxidase, using oxygen to convert the ferrous iron to the ferric state for deposition and storage. The stored ferric iron can be reduced back to the ferrous state and released from the ferritin molecule should iron be needed later by the mucosal or other nonintestinal cells. If not needed, the iron remains as ferritin and is excreted when the short-lived (2–3 days) mucosal cells are sloughed off into the lumen of the gastrointestinal tract. Ferritin synthesis in the intestine and other tissues is directly affected by iron, with increased synthesis associated with increased iron absorption and decreased synthesis associated with low iron absorption. Ferritin is described in further detail in the section on iron storage.

Iron moving through the mucosal cell may be used by the cell for a variety of functions, especially as a cofactor for enzymes. Iron that is not needed within the enterocyte may be released into the blood following transport across the basolateral (serosal) membrane.

Iron transport across the intestinal cell basolateral membrane requires binding to another membrane transport protein called ferroportin (Fp), also known as Ireg 1 or MTP 1. Transport of ferrous iron across the basolateral membrane is coupled with its oxidation to Fe³⁺ by a copper-containing protein called hephaestin. The oxidation of iron to the ferric state is essential for the transport of iron in the blood as part of transferrin. Transferrin is the main iron transport protein; it binds and carries up to two iron atoms (termed diferric) in the blood for iron transport to tissues.



Regulation of Iron Absorption One regulator of iron absorption is the protein hepcidin, which is released from the liver when body iron stores are adequate or high. The liver is thought to recognize the body's high or adequate iron situation, at least in part, by the binding of diferric transferrin to transferrin 2 receptors (TfR2) on liver cells. The subsequent uptake of diferric transferrin by transferrin receptors 2 in the liver is thought to stimulate hepcidin synthesis, although, another protein called HFE (and possibly hemojuvelin) also modulates hepatic hepcidin synthesis. Hepcidin, upon release from the liver, travels in the blood targeting enterocytes and macrophages, and hepcidin's interaction promotes the internalization and degradation of the protein ferroportin. Ferroportin is found on the basolateral membrane of mature enterocytes and on the cell membranes of macrophages. With the hepcidin-induced loss of ferroportin from the cell membranes, iron cannot be transported out of the enterocyte or out of the macrophage and thus cannot get into the blood for use by other tissues. Thus, increased hepcidin concentrations result in increased enterocyte and macrophage iron concentrations. In the case of the enterocyte, availability of newly absorbed iron to the body is decreased.

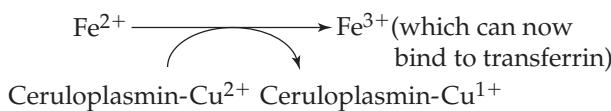
The enterocyte basolateral (serosal) membrane contains some additional proteins (besides ferroportin) involved in iron uptake and efflux and thus absorption. HFE, a histocompatibility class I-like protein, is present and appears to interact with transferrin receptors (TfR) to mediate transferrin-bound iron uptake across the basolateral membrane and into the enterocyte from the plasma. β 2-microglobulin is also present as part of the complex with HFE and TfR and stabilizes, transports, and expresses HFE. When body iron is high, uptake of iron from the plasma and into the intestinal cells is increased. Increases in iron in cells such as enterocytes affect the synthesis of other proteins involved in iron uptake. For example, increased iron in enterocytes causes diminished synthesis of proteins involved in iron absorption such as Dcytb and DMT1. Consequently, in times of adequate or high iron stores, iron absorption is diminished. Mutations, however, can inhibit normal regulatory mechanisms. Mutations in HFE, for example, can inhibit transferrin-bound iron uptake into the enterocyte from the plasma and diminish hepcidin synthesis in the liver, resulting in the iron toxicity disorder called hemochromatosis (see the "Toxicity" section). Similarly, in the absence of hepcidin (due to genetic defects), iron accumulates in toxic amounts, and in the presence of excessive hepcidin levels (due to genetic defects), iron deficiency occurs.

Conversely, when iron stores are low, the absence of or low levels of hepcidin, coupled with low iron uptake from the plasma into the enterocyte, result in the synthesis of proteins like Dcytb and DMT1 and of continued ferroportin expression in the membranes. Iron is then transported out of enterocytes as well as out of macrophages and into the blood so that the iron can be used by the body.

Transport

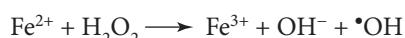
Iron in its oxidized ferric state is transported in the blood attached to the protein transferrin. Iron oxidation, transferrin's role in iron transport, and the importance of protein in iron binding in the body are reviewed next.

As mentioned in the previous section, iron must first be oxidized before it can bind to transferrin for transport in the blood. Hephaestin, found in the intestinal cells, and ceruloplasmin, found throughout the body, are both copper-containing proteins with ferroxidase activity. These proteins catalyze the oxidation of ferrous iron to its ferric form so it can bind to transferrin in the plasma. The role of copper as part of hephaestin and ceruloplasmin is crucial to iron metabolism. Copper deficiency results in iron accumulation in sites such as the intestine and liver and reduced iron transport to tissues. The role of ceruloplasmin in the oxidation of iron may be depicted as follows:



Transferrin, a glycoprotein made primarily in the liver, has two binding sites for minerals. The binding site near the carboxy (C)-terminal end of transferrin has a high affinity for ferric iron. The binding site near the amino (N)-terminal end has a high affinity for ferric iron but also binds other minerals, such as chromium, followed in descending order by copper > manganese > cadmium > zinc and nickel. The binding of ferric iron to transferrin requires the presence of an anion, usually bicarbonate, at each binding site. Transferrin in the plasma is typically about one-third (33%) saturated with ferric iron. If all of transferrin's binding sites were occupied (as occurs with toxicity), then the transferrin would be fully (100%) saturated.

The role of proteins in the transport as well as storage of iron is important because of iron's redox activity. The binding of iron by proteins serves as a protective mechanism. Left unbound, the iron's redox activity can lead to the generation of harmful free radicals. Free ferrous iron (Fe^{2+}), for example, readily reacts with hydrogen peroxide (H_2O_2) in a reaction known as the Fenton reaction:



This reaction generates a hydroxyl anion and a free hydroxyl radical ($\cdot\text{OH}$), which is extremely reactive and damaging

to cells (see the Perspective in Chapter 10). In addition, the binding of iron by protein is important to ensure that bacteria that may be present in the body, as with an infection, are unable to use the iron for their own (bacterial) growth. Free iron—but not protein-bound iron—is readily used by bacteria for proliferation and growth. Bacteria cannot multiply without nutrients such as iron, acquired from the host. Thus, keeping iron attached to proteins in the body diminishes bacterial multiplication.

Transferrin binds and transports not only newly absorbed dietary iron that has crossed the basolateral membrane of the mucosal cell, but also transports iron that has been released following the degradation of iron-containing compounds in the body. In fact, most of the iron entering the plasma for distribution by transferrin is contributed from hemoglobin destruction and release from storage.

Thus, transferrin ferries iron throughout the body, delivering both new and recycled iron to tissues either for use or for storage. Transferrin has a half-life of about 7 to 10 days.

Storage

Iron not needed in a functional capacity is stored in three main sites: the liver, bone marrow, and spleen. Transferrin delivers iron to these sites, especially the liver, which is

thought to store about 60% of body's iron. The remaining 40% is found in reticuloendothelial (RE) cells within the liver, spleen, and bone marrow (and possibly between muscle fibers). Most of the iron stored in reticuloendothelial cells is derived from phagocytosis of red blood cells and subsequent degradation of the hemoglobin within those cells.

Ferritin is the primary storage form of iron in cells. Ferritin is synthesized in a variety of tissues, especially within the liver, spleen, bone marrow, and intestine, and consists of apoferitin in which iron atoms have been deposited. Ferritin, which is shaped like a sphere (or apoferitin which is shaped like a hollow sphere), is composed of 24 protein subunits. Ferritin's subunits are classified based on molecular mass as H or L, and the proportions of H and L subunits within ferritin vary between tissues. The L form, for example, predominates in the liver and spleen and takes up iron rather slowly, compared with the H form. Iron enters apoferitin through channels or pores. The pores serve as the site of the oxidation of ferrous iron into ferric oxyhydroxide crystals ($4 \text{ Fe}^{2+} + \text{O}_2 + 6 \text{ H}_2\text{O} \rightarrow 4 \text{ FeOOH} + 8 \text{ H}^+$) or ferrihydrite ($5 \text{ Fe}_2\text{O}_3 + 9 \text{ H}_2\text{O}$), and molecular oxygen functions as the electron acceptor. Ferric oxyhydroxide or ferrihydrite is deposited in the interior of the protein shell (Figure 12.4). As many as 4,500 iron atoms can be stored in ferritin.

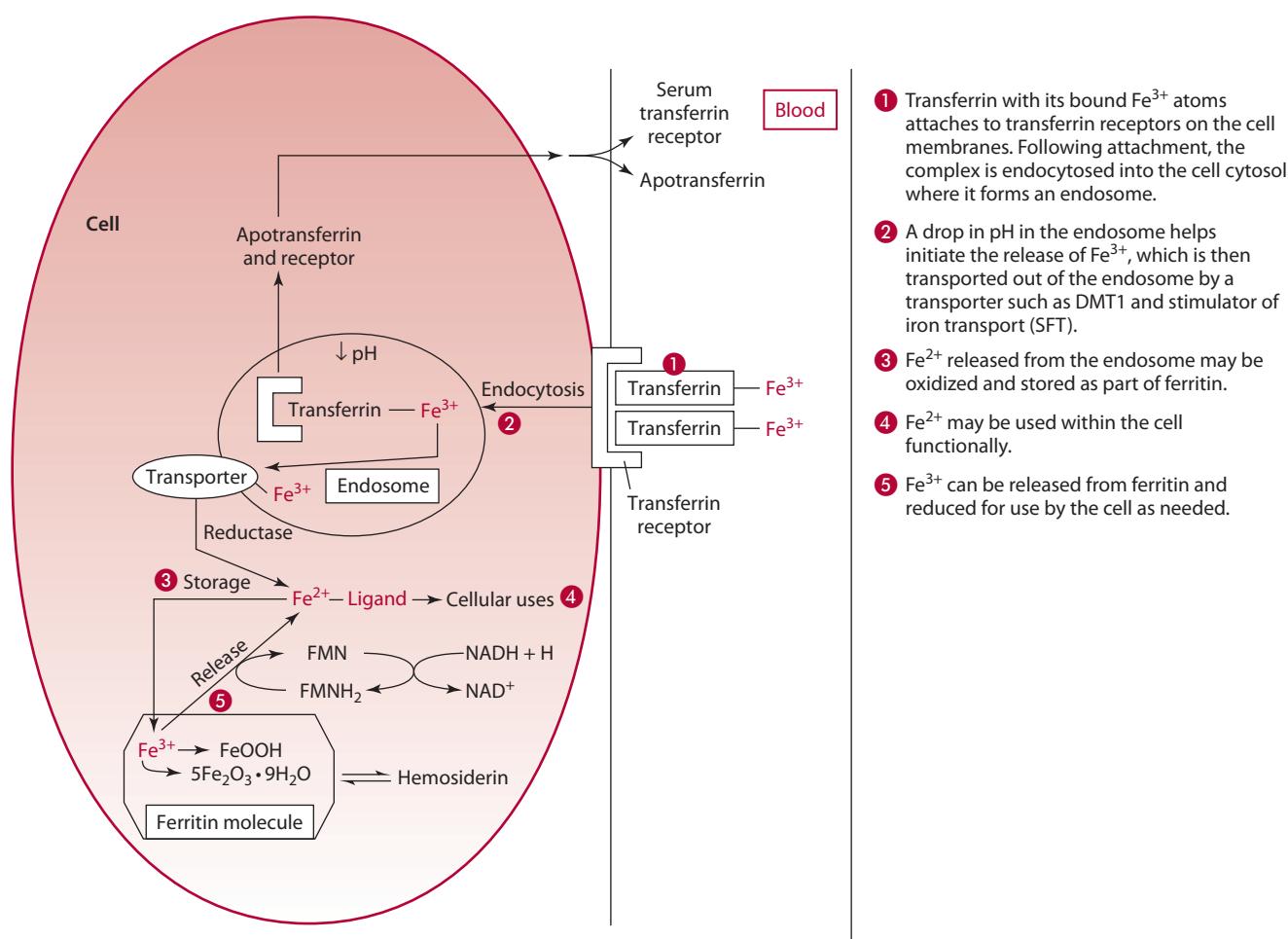


Figure 12.4 Overview of iron uptake and storage.

Ferritin is not a stable compound but rather is constantly being degraded and resynthesized, providing an available intracellular iron pool. Cellular iron is thought to influence in part the synthesis of ferritin at the translation level (Figure 12.5). Specifically, an iron regulatory response element binding protein (IRE-BP), also called an iron response protein (IRP) responds to the cell's iron status. This IRE-BP's ability to respond depends on the cell's iron status. With high amounts of iron, the IRE-BP exists as a 4Fe-4S cluster and exhibits aconitase activity. The aconitase functions in the mitochondria to convert citrate to isocitrate as part of the TCA cycle. In contrast, with less iron, the IRE-BP exists as a 3Fe-4S cluster and functions as a binding protein. As a binding protein, the IRE-BP binds to iron response elements (IREs) located in the 5' untranslated region of ferritin mRNA. (The IRE-BP, however, can also bind to other IREs located in the 3' untranslated region of mRNAs of other proteins such as TfR, Dcyt reductase, and DMT1). IREs are stem loop structures of about 30 nucleotides found in the mRNA. In low-iron situations, the IRE-BP acts as a binding protein and binds to the IRE in ferritin mRNA; this binding of IRE-BP to the 5' region of the ferritin mRNA acts as a repressor to inhibit the translation of the ferritin protein. Thus, less ferritin protein is made in cells when cellular iron content is low. From a physiological standpoint this inhibition makes sense, because ferritin stores iron, and

not much ferritin would be needed if the cell's iron content was low. Under the opposite conditions, in which the cell has a relatively high iron content, the IRE-BP (containing a 4Fe-4S cluster) exhibits aconitase activity. Without the binding of the IRE-BP (4Fe-4S), the ferritin mRNA undergoes translation. Thus, more ferritin protein is made in cells when cellular iron concentrations are high.

Equilibration occurs between tissue ferritin and serum ferritin. Thus, serum ferritin is used as an index of body iron stores: 1 ng ferritin/mL serum equals ~10 mg body iron stores. Normal serum ferritin concentrations (for adults) exceed ~12 ng/mL; however, because ferritin acts as an acute phase (reactant) protein, it is not a reliable indicator of iron stores during, and possibly for several weeks following, inflammation or illness. In other words, serum ferritin concentrations may be elevated or within the normal range in the blood, despite an individual's having little to no iron. Methods of assessing iron status are described further in the section "Assessment of Nutriture."

Hemosiderin is another iron storage protein. Hemosiderin is thought to be a degradation product of ferritin, representing, for example, aggregated ferritin or a deposit of degraded apoferritin and coalesced iron atoms. The content of iron in hemosiderin may be as high as 50%. The ratio of ferritin to hemosiderin in the liver varies according to the level of iron stored in the organ, with ferritin predominating at lower iron concentrations, and hemosiderin

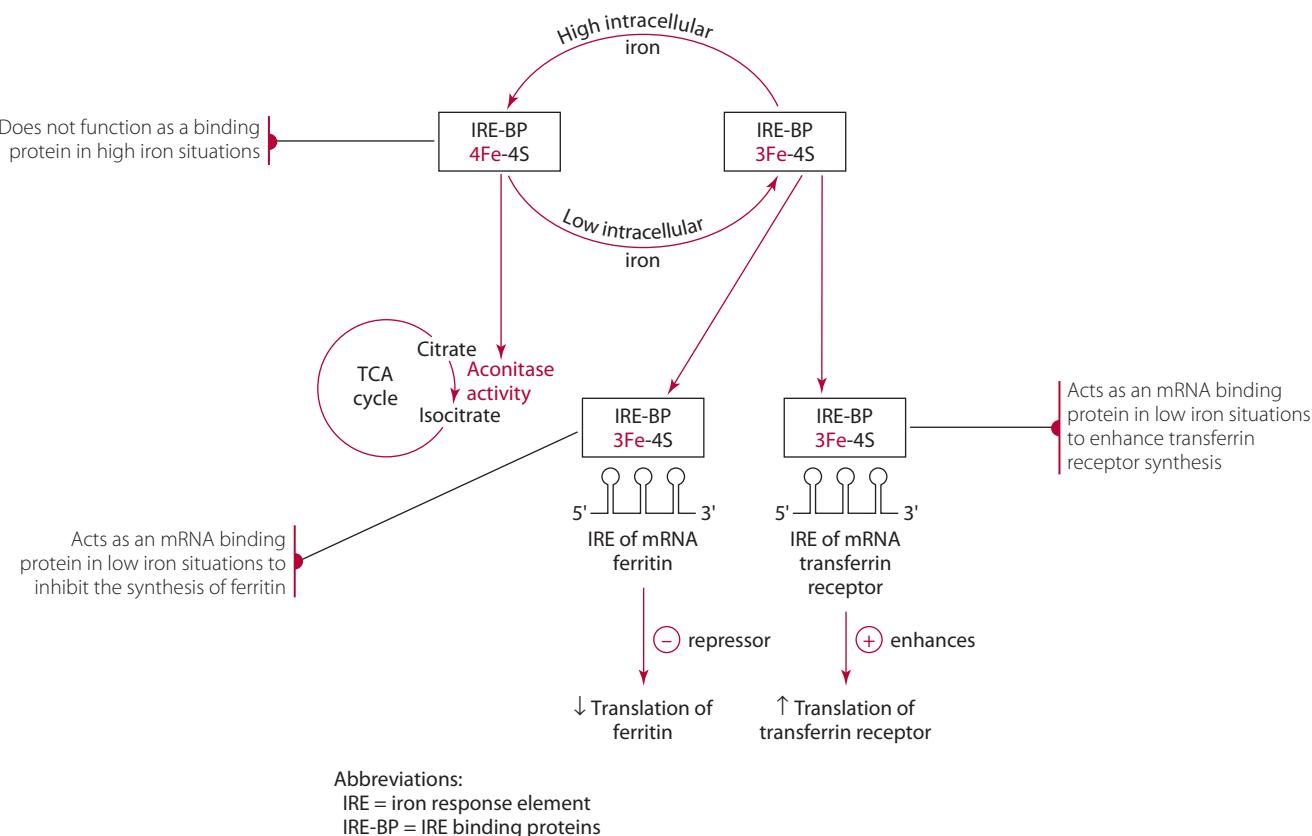


Figure 12.5 The influence of intracellular iron on the translation of ferritin mRNA and transferrin receptor mRNA.

predominating at higher concentrations (iron overload). Although iron in hemosiderin can be labilized to supply free iron, the rate at which iron is released from hemosiderin is slower than that from ferritin.

Release of iron from stores (Figure 12.4) requires mobilization of Fe^{3+} and the use of reducing substances such as riboflavin (FMNH_2), niacin (NADH), or vitamin C and possibly a chelator to enable diffusion through ferritin pores. However, following the reduction of iron to release it from storage, Fe^{2+} is transported to the cell surface, where it must be reoxidized to allow transport out of the cell. This reoxidation of iron to enable binding to transferrin for transport to tissues requires ceruloplasmin, as previously described in the section on iron transport. The superoxide radical (O_2^\bullet) also has been found to initiate iron release from ferritin in vitro. However, only one or two iron atoms from ferritin are released, even with extended exposure to superoxide radicals. The size and age of ferritin's iron core, not the iron content of ferritin's protein shell, affect iron release [18].

Uptake by Tissues

The amount of iron taken up by the tissues depends in part on the transferrin saturation level. For example, iron delivery is greater from diferric transferrin (transferrin containing two bound iron atoms) than from monoferric transferrin (transferrin containing only one bound iron atom). For iron uptake into tissues to occur, the transferrin molecule bound to iron (either diferric or monoferric transferrin) must first bind to transferrin receptors (TfRs) on cells (Figure 12.4). Transferrin receptors consist of two subunits that each bind one transferrin molecule. Liver and intestinal cells appear to contain an isoform of the transferrin receptor, called TfR2. Most other cells contain TfR1. TfR2 prefers to bind diferric transferrin.

For iron to be taken up into the cells, the transferrin molecule with the iron attached first binds to the receptor and forms a complex. The complex is thought to be internalized by endocytosis and to form a vesicle (also called an endosome) in the cytosol of the cell. Next, in an ATP-dependent process, protons are pumped into the endosome and reduce the pH to about 5.5. In the presence of the acidic pH and possibly other factors, iron atoms are released from the transferrin molecule. The apotransferrin is then thought to return to the cell surface and plasma. Use of the released iron requires its transport across the endosomal membrane. DMT1 (a transporter that is also found on the brush border membrane of the intestinal cells), along with a protein called stimulator of iron transport (SFT), is thought to transport the iron across the endosomal membrane.

The number of transferrin receptors on cells increases or decreases depending on intracellular iron concentrations. In other words, intracellular iron affects the genetic expression of transferrin receptors on the cell, as shown in

Figure 12.5. As with ferritin mRNA, mRNA for the transferrin receptor contains IREs. For the transferrin receptors, the IREs are in the 3' untranslated region (whereas for ferritin, the IREs were in the 5' region). Remember that IREs are stem loop structures of about 30 nucleotides found in the mRNA. Within the cytosol, again as with ferritin, IRE binding proteins (IRE-BPs), with multiple iron-sulfur clusters, respond to the cell's iron status. In a low-cellular-iron situation, the IRE-BP contains a 3Fe-4S cluster and readily binds to the IRE. When bound to the 3' region, the IRE-BP stabilizes the transferrin receptor mRNA. The stabilized transferrin receptor mRNA exhibits a longer half-life, and consequently more transferrin receptor mRNA is translated into transferrin receptor proteins. Once made, these transferrin receptor proteins become embedded in the cell's plasma membrane to promote cellular iron uptake. Thus, in conditions of low cellular iron, transferrin receptor synthesis is increased.

If the intracellular iron concentration is relatively high, fewer transferrin receptors are translated. With adequate or high cellular iron, the IRE-BP exists as a 4Fe-4S cluster and exhibits aconitase activity in the mitochondria, and thus does not act as a binding protein for the IRE of the transferrin receptor mRNA. Without the IRE-BP bound to the IRE of transferrin receptor mRNA, the mRNA is not as stable and is more quickly degraded. This decreased stability and increased degradation in turn diminish translation of the mRNA and result in fewer transferrin receptor proteins being produced. The synthesis of fewer transferrin receptor proteins means that fewer receptors are available on the cell surface, and less iron is brought into the cell. Thus, the level of transferrin receptor expression indicates the cell's need for iron uptake.

FUNCTIONS AND MECHANISMS OF ACTION

Iron functions in the body as part of several proteins, including serving as a cofactor for dozens of enzymes. In many body proteins, iron is present as part of heme. In other proteins, iron is found in a cluster with sulfur (2Fe-2S, 4Fe-4S, or 3Fe-4S), by itself as a single atom, or as part of a bridge with oxygen. Heme proteins represent the largest group and include hemoglobin, myoglobin, cytochromes involved in electron transport, and enzymes such as monooxygenases, dioxygenases, and oxidases. Iron sulfur proteins also include several enzymes involved in electron transport, as well as a few nonredox enzymes such as aconitase and ferrochelatase. Proteins that contain single iron atoms are mostly mono- and dioxygenase enzymes, and the one iron oxygen bridge protein also is an enzyme, ribonucleotide reductase.

Hemoglobin and Myoglobin

The essentiality of iron is due in part to its presence in heme, which functions as a prosthetic group for some

proteins. The atom of iron in the center of the heme molecule enables oxygen transport to tissues (hemoglobin); transitional storage of oxygen in tissues, particularly muscle (myoglobin); and transport of electrons through the respiratory chain (cytochromes).

Hemoglobin is synthesized in red blood cells and carries about 98.5% of the total oxygen found in the blood. Hemoglobin consists of a globin portion, which is made up of four polypeptides, and four heme groups. Each polypeptide chain is associated with one of the heme molecules. Heme is an iron-containing derivative of porphyrin. Porphyrins, in turn, are cyclic compounds made up of four pyrrole rings joined together by methenyl bridges. Nitrogen atoms in each of the four pyrrole rings bind to the iron atom (Figure 12.6), and these bonds hold the iron atom in the plane of the porphyrin ring. The iron atom in the center of the heme has two remaining coordinate bonds available for binding. One is with an amino acid (often the nitrogen atom of histidine) of the protein to which the heme is attached. For example, in hemoglobin, the iron in the heme binds to the nitrogen of an amino acid in the protein globin; heme is found in a hydrophobic pocket of the protein. The sixth and last coordinate bond in heme proteins that bind oxygen—namely, hemoglobin and myoglobin—exists between the iron and oxygen. The oxygen is held quite loosely so that transfer to tissues can be rapid. In heme proteins that do not bind oxygen, the sixth coordinate bond is with atoms of amino acid groups in the protein (such as an enzyme) with which the heme group is associated.

Heme synthesis and the attachment of globin occur primarily in the red blood cells of bone marrow. Heme synthesis accounts for the largest use of functional iron in the body. In fact, each red blood cell is thought to contain millions of hemoglobin molecules, and all the red blood cells in the body together contain about two-thirds of total body iron. Erythropoietic cells in bone marrow possess transferrin receptors on their cell surface. Transferrin delivers the iron for heme synthesis to the erythropoietic cells in the bone marrow. The synthesis of heme (Figure 12.6) occurs as follows:

- Heme synthesis begins in the mitochondria, where glycine and succinyl CoA combine to form Δ -aminolevulinic acid (ALA). The reaction is catalyzed by Δ -aminolevulinic acid synthase, a vitamin B_6 -dependent enzyme that is inhibited by the final end product (heme) and whose synthesis is also thought to be regulated by iron.
- Next, ALA enters the cytosol, where a zinc-dependent dehydratase catalyzes the condensation of two ALA molecules to form porphobilinogen. This enzyme is sensitive to lead, which binds to its sulfhydryl groups to inactivate the enzyme.
- Next, in a series of cytosolic reactions involving a deaminase, a synthase, and a decarboxylase, four

porphobilinogens condense to form a tetrapyrrole that cyclizes. Side chains are modified, and coproporphyrinogen III is formed and enters the mitochondria.

- Coproporphyrinogen is converted in the mitochondria to protoporphyrinogen.
- Protoporphyrinogen is oxidized to form protoporphyrin IX.
- Last, an iron (Fe^{2+}) atom is inserted into protoporphyrin IX to yield heme. The insertion of iron into the heme is catalyzed by ferrochelatase, a 2Fe-2S cluster protein. The transcription of the ferrochelatase enzyme appears to be regulated by iron.

Unlike hemoglobin, which is a tetrameric protein, myoglobin consists of a single hemoprotein chain. Myoglobin, which is found in the cytosol of the muscle cells, facilitates the diffusion rate of dioxygen from capillary red blood cells to the cytosol and mitochondria of muscle cells.

Cytochromes and Other Enzymes Involved in Electron Transport

Heme-containing cytochromes in the electron transport chain, such as cytochromes b and c, pass along single electrons. The transfer of electrons along the chain is made possible by the change in the oxidation state of iron. In the reduced cytochromes, the iron atom is in the ferrous state. The iron atom of the reduced cytochrome becomes oxidized to the ferric state when a single electron is transferred to the next cytochrome. The iron atom of the cytochrome receiving the electron then becomes reduced. Other heme iron containing cytochromes include cytochrome b₅ (involved in lipid metabolism) and the cytochrome P₄₅₀ family (involved in drug metabolism and steroid hormone synthesis).

Nonheme iron sulfur enzymes involved in electron transport include NADH dehydrogenase, succinate dehydrogenase, and ubiquinone–cytochrome c reductase. Whether iron is carrying oxygen or transporting electrons, its essentiality in energy transformation is without question.

Monooxygenases and Dioxygenases

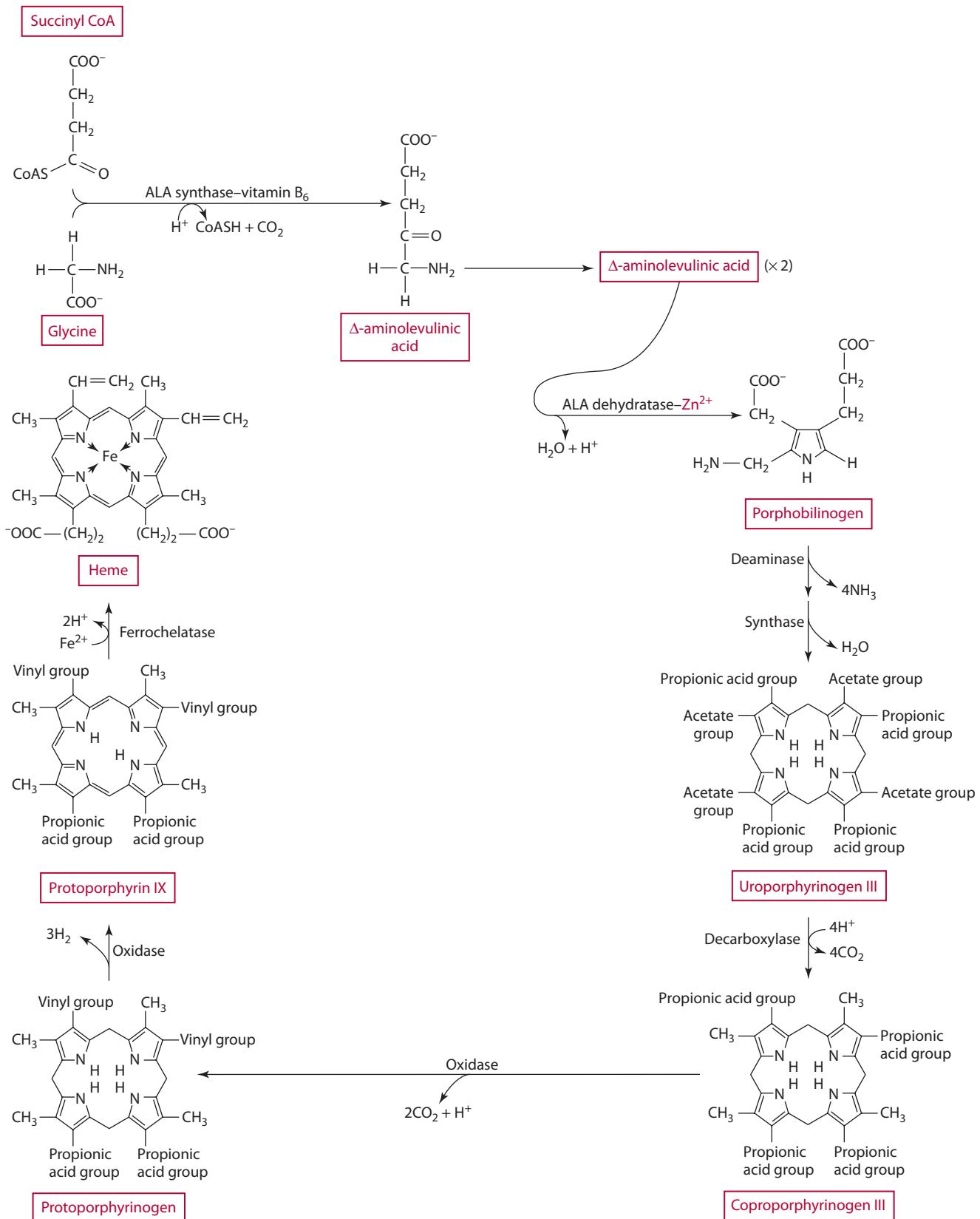
Many additional enzymes involved in a variety of processes besides the respiratory chain also require iron. Many monooxygenases, for example, need iron. Monooxygenases insert one of two oxygen atoms into a substrate. Examples of iron-containing monooxygenases include:

- phenylalanine monooxygenase
- tyrosine monooxygenase
- tryptophan monooxygenase

These enzymes insert an oxygen atom into the aromatic amino acids phenylalanine, tyrosine, and tryptophan,

Mitochondria

Cytoplasm



respectively. Monooxygenases also use cosubstrates to furnish the hydrogen atoms that reduce the second oxygen atom to water. Phenylalanine monooxygenase, tyrosine monooxygenase, and tryptophan monooxygenase all use tetrahydrobiopterin as a cosubstrate, and during the reactions tetrahydrobiopterin is oxidized to dihydrobiopterin. The reactions catalyzed by these three enzymes are shown in Figures 6.28 and 6.29.

Many dioxygenases also need iron. Dioxygenases catalyze the insertion of two oxygen atoms into a substrate. Many important dioxygenases in the body require iron, including:

- tryptophan dioxygenase (amino acid metabolism)
- homogentisate dioxygenase (amino acid metabolism)
- trimethyl lysine dioxygenase and 4-butyrobetaine dioxygenase (carnitine synthesis)
- lysine dioxygenase and proline dioxygenase (procollagen synthesis)
- nitric oxide synthase

Some of these reactions are covered in the following text. For example, tryptophan dioxygenase (a heme-containing enzyme, also called a pyrrolase) converts the amino acid tryptophan to N-formylkynurenine (Figure 6.29), representing the first step of tryptophan metabolism. Iron deficiency has been shown to reduce the efficacy of tryptophan as a precursor of niacin [19]. Normally, about 60 mg tryptophan can be converted to 1 mg niacin. Homogentisate dioxygenase is also involved in amino acid metabolism, specifically that of tyrosine. During tyrosine metabolism, tyrosine is transaminated to produce hydroxyphenylpyruvate, which is then converted to homogentisate. Homogentisate in turn is converted to 4-maleylacetoacetate by homogentisate dioxygenase, a single iron-dependent enzyme (Figure 6.28). Defects in this enzyme result in the genetic disorder alkaptonuria, which is characterized by high concentrations of homogentisate in the urine. When urine is excreted and the homogentisate is exposed to air, the compound turns a very dark color causing the urine to appear almost black. In those with alkaptonuria, the homogentisate also accumulates in joints, causing arthritis.

Two of the four steps required for carnitine synthesis involve iron-dependent dioxygenases. Recall that carnitine is an important nitrogen-containing compound necessary for the transport of long-chain fatty acids into the mitochondria for oxidation. The first step in carnitine synthesis (Figure 6.12), in which trimethyl lysine is converted to 3-OH trimethyl lysine, requires a single iron-containing trimethyl lysine dioxygenase, and the final step, in which 4-butyrobetaine is converted to carnitine, requires 4-butyrobetaine dioxygenase, another single iron-containing enzyme. α -ketoglutarate is a required cosubstrate in both of these reactions, and during

the reactions the keto acid becomes oxidatively decarboxylated to succinate. Vitamin C also participates in the reactions as a reducing agent.

Hydroxylation reactions for procollagen synthesis are shown in Figure 9.4. Both lysine and proline dioxygenases contain single iron atoms. As described in the steps for carnitine synthesis, α -ketoglutarate is a required cosubstrate, and vitamin C serves as a reducing agent.

Two isoforms of nitric oxide synthase, a dioxygenase needed for the synthesis of nitric oxide (a potent biological effector molecule), require heme iron.

Peroxidases

Other important reactions required to protect the body also involve iron-containing enzymes.

- Catalase, with four heme groups, converts hydrogen peroxide to water and molecular oxygen:

$$2 \text{ H}_2\text{O}_2 \longrightarrow 2 \text{ H}_2\text{O} + \text{O}_2$$

Catalase thus helps prevent cellular damage that can be induced by hydrogen peroxide (see the Perspective in Chapter 10).
- Myeloperoxidase (also called chloroperoxidase), another heme-containing enzyme, is found in the plasma as well as in neutrophils (white blood cells). During phagocytosis of bacteria, myeloperoxidase is released into the phagocytic vesicle within the neutrophil. The phagocytic vesicle contains a variety of compounds, including hydrogen peroxide (H_2O_2), free hydroxyl radicals ($\cdot\text{OH}$), and other ions such as chloride (Cl^-). Myeloperoxidase catalyzes the following reaction:



The OCl^- (hypochlorite) formed in the reaction is a strong cytotoxic oxidant that is important in destroying foreign substances, such as bacteria. The activity of myeloperoxidase may be impaired with iron deficiency, resulting in increased susceptibility to or severity of infection. Peroxidases also are important in producing thyroid hormones.

- Thyroperoxidase, a heme-dependent enzyme, is necessary for organification of iodide (a process in which 2 iodides (I^-) are added to tyrosine residues on thyroglobulin). The same enzyme then also conjugates the thyroglobulins (see the section “Functions and Mechanisms of Action” in the “Iodine” portion of this chapter). These reactions are necessary for the synthesis of the thyroid hormones T_3 and T_4 . Iron deficiency, in fact, is associated with decreased thyroperoxidase activity resulting in decreased T_3 and T_4 synthesis [20].

Oxidoreductases

Some oxidoreductases that are iron (and also molybdenum) dependent include:

- aldehyde oxidase, which uses oxygen to convert aldehydes (RCOH) to alcohols (RCOOH)

- sulfite oxidase, an iron sulfur-containing enzyme that converts sulfite (SO_3^-) to sulfate (SO_4^{2-})
- xanthine oxidase and dehydrogenase, both iron sulfur cluster enzymes that convert hypoxanthine generated from purine catabolism to xanthine and then convert xanthine to uric acid for excretion (Figure 12.23 in the "Molybdenum" section of this chapter). Remember that purine bases are found in DNA.

Other Iron-Containing Proteins

Another iron-dependent enzyme involved in DNA synthesis, and thus cell replication, is ribonucleotide reductase, which converts adenosine diphosphate (ADP) into deoxy ADP (dADP). This enzyme contains iron as part of a bridge with oxygen ($\text{Fe}^{3+} - \text{O}_2 - \text{Fe}^{3+}$). In glycolysis, glycerol phosphate dehydrogenase, a flavoprotein, has a nonheme iron component. In addition, phosphoenolpyruvate (PEP) carboxykinase, important in gluconeogenesis, also requires iron for its functioning.

Iron as a Pro-oxidant

As a pro-oxidant, free ferrous iron may catalyze the nonenzymatic Fenton reaction



In this reaction, ferrous iron reacts with hydrogen peroxide to generate ferric iron and the free hydroxyl radical ($\cdot\text{OH}$). In a reaction known as the Haber Weiss reaction, the superoxide radical, O_2^- , reacts with hydrogen peroxide to generate molecular oxygen and free hydroxyl radicals ($\cdot\text{OH}$). Hydroxyl radicals are dangerous membrane oxidants.



INTERACTIONS WITH OTHER NUTRIENTS

You have read that iron and ascorbic acid interact, enhancing iron absorption and maintaining iron in the appropriate valence state for enzyme function. The potential also may exist for vitamin C-induced release of ferric iron from ferritin, with subsequent reduction of iron to the ferrous form [21]. Whether such reactions result in Fenton reactions and occur *in vivo* is unclear.

An interrelationship also exists between iron and copper because of the role of the copper-containing hephaestin and ceruloplasmin as a ferroxidase. In the 1920s, studies revealed that iron therapy was unable to cure anemia in rats; however, ashed foodstuffs containing copper replenished blood hemoglobin concentrations [22]. Without copper-dependent ferroxidase activity, iron cannot be mobilized out of tissues, and the copper deficiency causes iron deficiency anemia.

Another nutrient with which iron appears to interact is zinc. Ingesting both nutrients as a 25:1 molar ratio of

nonheme iron (ferrous sulfate) to zinc diminished the absorption of zinc from water to 34%; however, when the same ratio of iron to zinc was given with a meal, no inhibitory effects were demonstrated [23]. Ratios of nonheme iron to zinc of 2:1 and 3:1 also have been shown to inhibit zinc absorption, although similar ratios of heme iron to zinc had no effect on zinc absorption [24]. Thus, excessive intake of nonheme iron, as may occur with supplements, may have a detrimental effect on zinc absorption.

Another association is that between vitamin A and iron. Reduced vitamin A status causes iron accumulation in selected organs such as the spleen and liver. Inadequate vitamin A status also is associated with altered red blood cell morphology, and decreased plasma iron and blood hemoglobin and hematocrit. The interaction between iron and vitamin A appears to be mediated at least in part through erythropoietin, a hormone made in the kidneys that stimulates erythropoiesis (red blood cell production). Specifically, vitamin A as retinoic acid binds to a response element on the gene for erythropoietin and stimulates erythropoietin synthesis. Thus, with insufficient vitamin A, the erythropoietin gene is not transcribed adequately. Red blood cell synthesis is diminished, and iron remains in stores. Supplementation of vitamin A in people with poor vitamin A and iron status increases erythropoietin synthesis and increases iron release from stores to provide the iron that is needed for erythropoiesis [25]. Another possible means by which vitamin A may influence iron is through the role of retinoic acid in the transcription of transferrin receptor genes in selected cells [26].

Iron and lead also interact. Lead inhibits the activity of Δ -aminolevulinic acid dehydratase, a zinc-dependent enzyme required in heme synthesis. Lead also inhibits the activity of ferrochelatase, the enzyme that incorporates iron into heme. Thus, lead poisoning is associated with iron deficiency. In addition, increased absorption of lead occurs with iron deficiency in animals and could be problematic for children, who are often iron-deficient and may have increased exposure to lead [27]. The mechanism by which iron deficiency improves lead absorption is unknown, but it may involve uptake through common divalent metal/cation transporters such as DMT1.

Iron deficiency is associated with decreased selenium concentrations as well as with decreased glutathione peroxidase synthesis and activity [28–30]. Glutathione peroxidase, a selenium-requiring enzyme, catalyzes the reduction of hydrogen peroxide and organic peroxides. The mechanism or mechanisms by which iron deficiency impairs selenium concentrations and the activity of selenium-dependent enzymes is not known. Iron is thought to be involved in the pretranslational regulation of the glutathione peroxidase synthesis. Alternately, iron deficiency may affect selenium absorption or increase selenium use in the body.

Turnover

Although dietary iron is important in maintaining the long-term adequacy of body iron, the amount of iron absorbed (about 0.06% of the total body iron content) cannot meet the daily iron needs of the body. Rather, avid conservation and constant recycling of body iron ensure an adequate supply.

Most of the iron entering the plasma for distribution or redistribution by transferrin results from hemoglobin, ferritin, and hemosiderin degradation (Figure 12.7). Hemoglobin is degraded primarily by phagocytes of the reticuloendothelial system (found in the liver, spleen, and bone marrow). Iron stored as ferritin and hemosiderin is degraded primarily in the liver, spleen, and bone marrow. Ferritin degradation is covered in the section on iron storage. Briefly, hemoglobin degradation occurs in this way: Most old (senescent) red blood cells, which live for about 120 days, are taken up by macrophages in the spleen and degraded (phagocytosed); however, reticuloendothelial cell macrophages in bone marrow and Kupffer cells in the liver also may degrade the red blood cells. During red blood cell degradation, the heme portion of the hemoglobin molecule in the red blood cell is catabolized by heme oxygenase to biliverdin and subsequently to bilirubin, which is then secreted into the bile for excretion from the body. With the degradation of heme, ~20 to 25 mg iron per day is made available. Ferroportin, the same protein responsible for iron efflux from intestinal cells, enables the transport of iron out of the macrophages. Specifically, ferroportin facilitates the transport of iron into vesicles in the macrophages, from which it is subsequently secreted into the blood. This process is facilitated by low hepcidin

concentrations. Iron released from the macrophages may be reused, for example, for erythropoiesis or for incorporation into iron-dependent enzymes, or the iron may be deposited for storage. In situations with increased hepcidin (as would occur with increased body iron), ferroportin is degraded, and iron is retained with the macrophages. Hepcidin concentrations are also elevated, however, with inflammatory conditions or infections, because of cytokine-induced hepcidin synthesis.

Although most red blood cells are degraded in the reticuloendothelial system, some (up to ~10%) red blood cell lysis occurs within the blood. Two proteins, haptoglobin and hemopexin, remove the released hemoglobin and any free heme, respectively, from the blood. Haptoglobin, synthesized by the liver, forms complexes with free hemoglobin, and hemopexin, also synthesized in the liver, forms a complex with free heme in the blood. The proteins then deliver the iron-containing compounds to the liver, where further degradation occurs to enable reuse of the iron. With significant hemolysis, the quantity of iron passing through the plasma can expand to six to eight times the normal amount. In contrast, should erythropoiesis decline dramatically, as occurs on descent from high altitudes, the quantity of iron in the plasma pool may decrease to as little as one-third of normal. Figure 12.7 represents schematically the internal iron exchange in the body.

EXCRETION

Daily iron losses for an adult male are ~0.9 to 1.0 mg/day (12–14 mg/kg/day). Iron losses for women (postmenopausal) are a bit lower, ~0.7 to 0.9 mg/day, because of

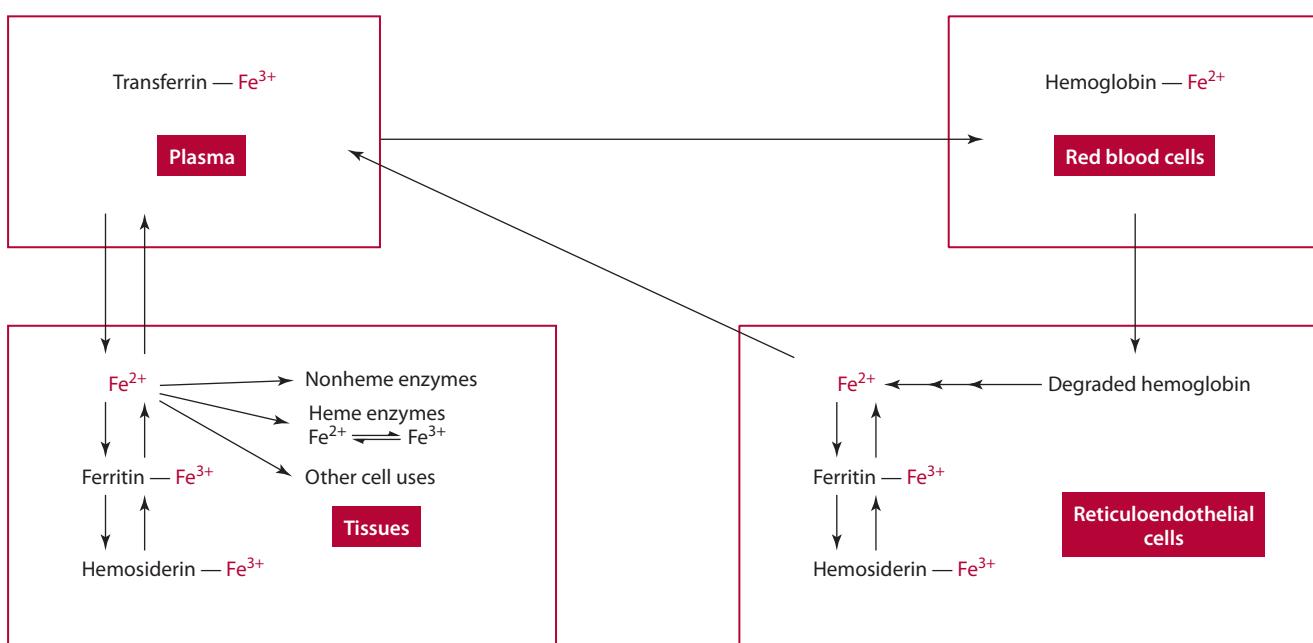


Figure 12.7 Internal iron exchange.

women's smaller surface area. Losses of iron occur from three main sites:

- the gastrointestinal tract
- the skin
- the kidneys

Of these sites, most (0.6 mg) iron losses occur through the gastrointestinal tract. Of the 0.6 mg, about 0.45 mg is lost through minute (~1 mL) blood loss (which occurs even in healthy people), and another 0.15 mg is through losses in bile and desquamated mucosal cells. The skin losses of ~0.2 to 0.3 mg iron occur with desquamation of surface cells from the skin. Finally, a very small amount, about 0.08 mg, is lost in the urine. Losses of iron, however, may be greater in people with gastrointestinal ulcers or intestinal parasites, or with hemorrhage induced by surgery or injury.

Total iron losses of premenopausal women are estimated to be ~1.3 to 1.4 mg/day because of iron loss in menses. The average loss of blood during a menstrual cycle is ~35 mL, with an upper limit of ~80 mL. The iron content of blood is ~0.5 mg/100 mL of blood, which translates into a loss of nearly 17.5 mg iron per period. Averaged out over a month, iron loss in menses is ~0.5 mg/day; in some women, however, iron loss during menses alone may exceed 1.4 mg/day. Balancing iron losses from the body with iron absorption is very important to health. Iron deficiency remains one of the most common nutritional deficiencies worldwide.

RECOMMENDED DIETARY ALLOWANCE

For adult men, the requirement and RDA for iron are 6 mg/day and 8 mg/day, respectively. For postmenopausal women, the requirement and RDA for iron are 5 mg/day and 8 mg/day, respectively [31]. Because of the greater losses associated with menses, premenopausal women require 8.1 mg iron/day; the recommended intake is 18 mg/day [31]. During pregnancy, though no menstrual losses occur, iron is needed for the fetus, for expanding blood volume, and for tissue and storage such that the RDA for iron is 27 mg/day. The RDA for iron is 9 mg/day during lactation [31]. The inside cover of the book provides additional RDAs for iron for other age groups.

DEFICIENCY: IRON DEFICIENCY WITH AND WITHOUT ANEMIA

Iron deficiency occurs most often due to inadequate iron intake. Iron intake is frequently inadequate in four population groups:

- infants and young children (6 months to about 4 years), because of the low iron content of milk and other

preferred foods, rapid growth rate, and insufficient body reserves of iron to meet needs beyond about 6 months

- adolescents in their early growth spurt, because of rapid growth and the needs of expanding red blood cell mass
- females during childbearing years, because of menstrual iron losses
- pregnant women, because of their expanding blood volume, the demands of fetus and placenta, and blood losses to be incurred in childbirth

In addition, many nonpregnant females during childbearing years fall short of the RDA for iron because of restricted energy (caloric) intake and inadequate consumption of iron-rich foods.

The need for iron is increased in other conditions and populations because of increased iron losses or impaired iron absorption. Conditions associated with increased iron losses include hemorrhage, renal disease, renal replacement therapy, decreased (faster than normal) gastrointestinal transit time, steatorrhea, and parasites. Impaired iron absorption may occur with protein energy malnutrition, renal disease, achlorhydria (the absence of hydrochloric acid in gastric juice), prolonged use of alkaline-based drugs such as antacids, and parasites.

Figure 12.8 depicts the gradual depletion of iron content in the body and demonstrates the fact that anemia does not occur until iron depletion is severe. Iron deficiency can occur without anemia, however. Symptoms of iron deficiency, mostly demonstrated in children, include pallor, listlessness, behavioral disturbances, impaired performance in some cognitive tasks, some irreversible impairment of learning ability, and short attention span [32]. In adults, work performance and productivity are most commonly impaired with iron deficiency [33,34]. Iron deficiency may impair the degradation of γ -aminobutyric acid (GABA), an inhibitory neurotransmitter in the brain, or may inhibit dopamine-producing neurons [32]. Possible impairment of the immune system, decreased resistance to infection, and impaired capacity to maintain body temperature have also been shown [32].

Further details about iron deficiency with and without anemia as it relates to changes that occur in indices of iron status are covered in the section "Assessment of Nutriture."

SUPPLEMENTS

Oral supplements of ferrous iron are available in complexes with sulfate, succinate, citrate, lactate, tartrate, fumarate, and gluconate. Intravenous administration of iron dextran also can be given. Oral iron supplements provide nonheme iron, and thus absorption of the iron is enhanced when ingested with a source of vitamin C or other enhancing factors. Amino-acid iron chelates, such as iron glycine, are also marketed; however, iron administered as a

	Normal	Early Negative Iron Balance	Iron Depletion	Iron-Deficient Erythropoiesis	Iron Deficiency Anemia	
Iron stores	High	Medium	Low	Very low	Very low	
Circulating iron	High	Medium	Low	Very low	Very low	
Erythron iron	High	Medium	Low	Very low	Very low	
Reticuloendothelial marrow iron	2–3 ⁺	1 ⁺	0–1 ⁺	0	0	
Transferrin iron-binding capacity (μg/dL)	330±30	330–360	360	390	410	
Plasma ferritin (μg/L)	100±60	<25	20	10	<10	
Iron absorption (%)	5–10	10–15	10–15	10–20	10–20	
Plasma iron (μg/dL)	115±50	<120	115	<60	<40	
Transferrin saturation (%)	35±15	30	30	<15	<15	
Sideroblasts (%)	40–60	40–60	40–60	<10	<10	
Erythrocyte protoporphyrin (μg/dL)	30	30	30	100	200	
Erythrocytes	Normal	Normal	Normal	Normal	Microcytic Hypochromic	
Serum transferrin receptors	Normal	Normal–high	High	Very high	Very high	
Ferritin iron	Normal	Normal–low	Low	Very low	Very low	

Figure 12.8 Sequential changes in iron status associated with iron depletion.

chelate has not been shown to be absorbed better than iron given as ferrous sulfate or ferrous ascorbate [35–37]. Initial effects of oral iron supplements on red blood cell counts and hemoglobin concentrations take about 2 weeks. Iron therapy to build up body stores of iron may be needed for 6 months to 1 year.

TOXICITY: HEMOCHROMATOSIS

Accidental iron overload (toxicity) has been observed in young children following excessive ingestion of iron pills or vitamin/mineral pills. Other people susceptible to iron overload have a genetic disorder known as *hemochromatosis*. An estimated 50 per 10,000 people in the United States are homozygous for the disorder. Hemochromatosis is most often seen in Caucasian males and becomes evident around 20 years of age. The condition is characterized by increased (at least two times normal) iron absorption. Mutations in the HFE gene cause the condition and result in the inability of the body, primarily the intestinal cells, to accurately sense iron stores and down-regulate intestinal iron absorption. In the C282Y mutation in HFE, tyrosine is substituted for cysteine because of a single base change. This mutation disrupts a disulfide bond in HFE that affects its interaction with $\beta 2$ -microglobulin and with transferrin receptors. Remember, these interactions are needed for cellular iron uptake and, in turn, down-regulation of iron absorption. Functional HFE is also needed to stimulate hepatic synthesis of hepcidin, which tells the intestinal

cells to down-regulate iron absorption. Although several mutations can cause some form of hemochromatosis, in most people with hemochromatosis iron absorption generally continues, despite high iron stores. The absorbed iron is progressively deposited within joints and tissues, especially the liver, heart, and pancreas, causing extensive organ damage and ultimately organ failure. Iron deposition in the liver, for example, leads to cirrhosis, usually by about 50 years of age. Heterozygotes for the condition do not develop severe organ dysfunction but do have abnormal iron status. Treatment of hemochromatosis requires frequent phlebotomy (removal of blood), usually the weekly removal of about 1 unit (~400 to 500 mL) of blood, which contains about 200 to 250 mg iron. In addition, deferoxamine may be given. Deferoxamine works by chelating (binding to) iron in the body and increasing urinary iron excretion. Treatment of hemochromatosis usually continues as described until serum ferritin concentrations are less than about 20 to 50 $\mu\text{g}/\text{L}$, and transferrin saturation is less than about 30% [38]. Once these levels are achieved, the frequency of phlebotomy usually diminishes.

Other people at particularly high risk for iron overload are those with iron-loading anemias, thalassemia, and sideroblastic anemia. The elevated erythropoiesis in people so affected causes increased iron absorption. However, although studies once linked high body iron (serum ferritin $>200 \mu\text{g}/\text{L}$) to heart disease, a larger and more recent group of studies has shown no such association.

ASSESSMENT OF NUTRITION

Numerous measurements are used to assess iron nutrition. The most common indices are hemoglobin (amount of iron-containing protein found in red blood cells per unit, usually deciliter or liter, of blood) and hematocrit (that proportion of the total blood volume that is red blood cells). However, although these indices indicate the presence of anemia, they are among the last to change as iron deficiency develops.

In the first stages of iron deficiency, iron stores in the liver, spleen, and bone marrow are diminished. Although iron stores can be aspirated and measured from bone marrow, the routine test involves measurement of plasma ferritin. Decreases in plasma ferritin concentration are thought to parallel the decrease in the amount of iron found in stores. Plasma ferritin concentrations $<\sim 12 \mu\text{g/L}$ are associated with iron deficiency. However, if inflammation or infection is present, serum ferritin concentration rises, an occurrence unrelated to iron stores. Thus, serum ferritin may appear within normal range or high while the body's iron status is quite low.

As iron deficiency progresses into the second stage, iron stores typically remain low, and transport iron decreases. Thus, plasma ferritin concentrations continue to be diminished, and circulating iron begins to decrease. Iron circulates in the blood bound to transferrin. With deficiency, transferrin saturation decreases to $<16\%$. (Remember that transferrin is normally about 33% saturated.) Transferrin saturation can be calculated by multiplying the serum iron concentration by 100 and then dividing by the total iron-binding capacity (TIBC). TIBC represents the amount of iron that plasma transferrin can bind and normally ranges from ~ 250 to $400 \mu\text{g/dL}$. Levels >400 suggest iron deficiency. Serum iron concentrations, which represent the amount of iron bound to transferrin, also are affected with iron deficiency, decreasing to $<\sim 50 \mu\text{g/dL}$ (normal values range from ~ 50 – $165 \mu\text{g/dL}$).

As circulating iron diminishes, functional or cellular iron also becomes limited. With diminished iron, free protoporphyrin concentrations in erythrocytes rise. Protoporphyrin is a precursor of heme (for hemoglobin) and accumulates within red blood cells when iron is not available. Erythrocyte protoporphyrin levels $>70 \mu\text{g/dL}$ red blood cells are associated with iron deficiency. In iron deficiency, the number of transferrin receptors on the cell surface, especially of immature red cells, also increases. The increased receptor number represents an up-regulation to enable cells to better compete for transferrin-bound iron. With iron deficiency, concentrations of serum transferrin receptors (sTFR), truncated forms of the membrane receptor protein, increase to $>8.5 \text{ mg/L}$ and are thought to be directly proportional to the functional tissue (that is cellular) iron deficit after depletion of iron stores.

In the final stages of iron deficiency, anemia occurs. Blood hemoglobin concentrations of $<12 \text{ g/dL}$ and

$<13 \text{ g/dL}$ for females and males, respectively, are suggestive of iron deficiency anemia. Hematocrit concentrations of $<37\%$ and $<40\%$ for women and men, respectively, also are typical of iron deficiency anemia. Characterization of red blood cells with respect to size (mean corpuscular volume, or MCV) and amount of hemoglobin they contain (mean corpuscular hemoglobin, or MCH, and mean corpuscular hemoglobin concentration, or MCHC) typically shows that they are lower than normal in the final stages of iron deficiency anemia. Descriptions of these assessments follow.

- MCV (fl) represents the size of the red blood cell. It is calculated by dividing hematocrit by red blood cells and then multiplying by 10.
- MCH (pg/rbc) represents the average hemoglobin content of each individual red blood cell. It is calculated by dividing hemoglobin by red blood cells and then multiplying by 10.
- MCHC represents the amount of hemoglobin in grams per deciliter (%) of red blood cells. It is calculated by dividing hemoglobin by hematocrit and then multiplying by 100.

In summary, red blood cells are pale (hypochromic) and small (microcytosis) with iron deficiency anemia. Figure 12.8 illustrates the changes that occur in the various measurements.

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Web Site

www.cfsan.fda.gov

Zinc

The human body contains ~1.5 to 2.5 g of zinc. Zinc is found in all organs and tissues (primarily intracellularly) and in body fluids. Zinc, a metal, can exist in several different valence states, but it is almost universally found as the divalent ion (Zn^{2+}).

SOURCES

Zinc is found in foods complexed with amino acids that are part of peptides and proteins and with nucleic acids. The zinc content of foods varies widely (Table 12.3). Very good sources of zinc are red meats (especially organ meats) and seafood (especially oysters and mollusks). Animal products are thought to provide between 40% and 70% of zinc consumed by most people in the United States. Other good animal sources of zinc include poultry, pork, and dairy products. Whole grains (especially bran and germ) and vegetables (leafy and root) represent good plant sources of zinc. Fruits and refined cereals are poor zinc sources. Plant sources not only have a lower zinc content, but zinc from plants is also absorbed to a lesser extent than zinc from meat [1].

Processing of certain foods may affect the zinc available for absorption. Heat treatment can cause zinc

Table 12.3 Zinc Content of Selected Foods

Food/Food Group	Zinc (mg/100 g)
Seafood	
Oysters	17–91
Crabmeat	3.8–4.3
Shrimp	1.1
Tuna	0.5–0.8
Meat and poultry	
Liver	3.1–3.9
Chicken	1.0–2.0
Beef, ground	3.9–4.1
Veal	3.1–3.2
Pork	1.6–2.1
Eggs and dairy products	
Eggs	1.1
Milk	0.4
Cheeses	2.8–3.2
Legumes (cooked)	0.6–1.0
Grains and cereals	
Rice and pasta (cooked)	0.3–0.6
Bread (wheat)	1.0
Bread (white)	0.6–0.8
Vegetables	0.1–0.7
Fruits	<0.1

Source: www.nal.usda.gov/fnic/foodcomp.

in food to form complexes that resist hydrolysis, thereby making zinc unavailable for absorption. Maillard reaction products—that is, amino acid–carbohydrate complexes resulting from browning, for example—are particularly notable for inhibiting zinc's availability for absorption.

In addition to dietary food sources, endogenous sources of zinc are provided by pancreatic and biliary secretions that are released into the gastrointestinal tract. Carboxypeptidase, for example, is a zinc metalloenzyme. Following carboxypeptidase activity, the enzyme itself is hydrolyzed and zinc is released. The released zinc is then available for absorption and reuse in the body.

DIGESTION, ABSORPTION, TRANSPORT, UPTAKE, AND STORAGE

Digestion

Zinc, like iron, needs to be hydrolyzed from amino acids and nucleic acids before it can be absorbed. Zinc is believed to be liberated from food during the digestive process, most likely by proteases and nucleases in the stomach and small intestine. Hydrochloric acid also appears to play an important role in zinc digestion and absorption. Antacids, H₂ receptor blockers (such as Zantac, Tagamet, or Pepcid), and proton pump blockers (such as Prevacid or Prilosec) increase gastric pH, resulting in decreased zinc absorption [2]. The role of gastric acid in zinc digestion and absorption has not been elucidated but may be related to impaired hydrolysis of zinc from nucleic or amino acids, changes in zinc's ionic state, or alterations in the enterocyte membrane to affect zinc absorption.

Absorption

The main site of zinc absorption in the gastrointestinal tract is the proximal small intestine, most likely the jejunum. However, the relative contribution of each segment of the small intestine (duodenum, jejunum, and ileum) toward overall zinc absorption has not been demonstrated. Zinc is absorbed into the enterocyte by a carrier-mediated process, with low zinc intakes absorbed more efficiently than higher intakes. A protein carrier called Zrt- and Irt-like protein (ZIP)4 is thought to be the primary transporter of zinc across the brush border membrane of the enterocyte as shown in Figure 12.9 [3,4]. ZIP is not thought to require ATP, but its mechanism of transport is not well delineated. However, a mutation in ZIP4 is known to cause the disorder acrodermatitis enteropathica. The condition is characterized by poor zinc absorption and is clinically manifested by skin lesions (which often become infected), especially on the face, knees, and buttocks; impaired growth; and low plasma zinc concentrations, representing signs and symptoms of zinc deficiency. High doses of zinc that can be absorbed by other means, especially by diffusion or paracellularly, typically correct the symptoms. Another transporter, DMT1 (divalent mineral transporter 1, but

also sometimes called divalent cation transporter—DCT—and hereafter called DMT1), was once thought to be involved in brush border zinc uptake. However, although zinc appears to up-regulate DMT1 mRNA expression, the DMT1 transporter does not appear to transport significant quantities of zinc into intestinal cells [5].

In addition to carrier-mediated transport, passive diffusion and paracellular zinc absorption also are thought to occur with high zinc intake. Studies have generally shown that zinc absorption varies from approximately 10% to 59%; at higher intake absorption diminishes, and at lower intake absorption increases. Zinc ingested in aqueous form and in amounts greater than 20 mg does not appear to be well absorbed [6].

Factors Influencing Zinc Absorption

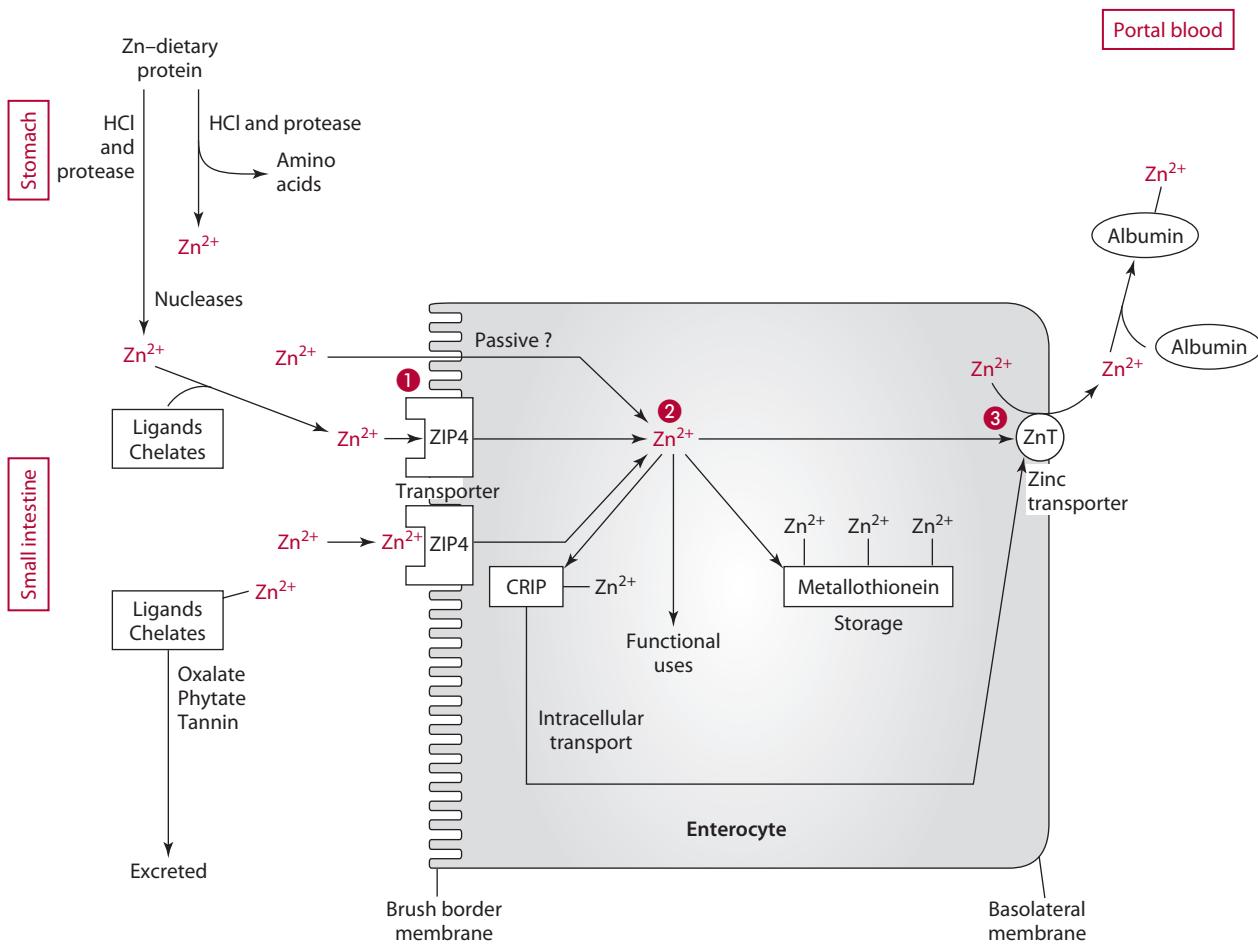
As is the case with iron, chelators or ligands may bind to zinc. Whether these substances are enhancers or inhibitors of zinc absorption depends on the digestibility and absorbability of the zinc chelates formed.

Enhancers of Zinc Absorption Several endogenous substances are thought to serve as ligands for zinc. Some of these ligands include citric acid, picolinic acid, and prostaglandins. Amino acid ligands include histidine, cysteine, and possibly lysine and glycine. Pancreatic secretions are thought to contain an unidentified constituent that enhances zinc absorption. In addition, glutathione (a tripeptide composed of cysteine, glutamate, and glycine) or products of protein digestion, such as tripeptides, are purported to serve as ligands. In these ligands, zinc typically binds to sulfur (e.g., cysteine alone, or as part of glutathione) or nitrogen (e.g., histidine). Ligands such as amino acids help maintain zinc's solubility in the gastrointestinal tract; whether zinc bound to amino acid ligands can be absorbed using amino acid transporters is unclear.

Absorption of zinc also appears to be enhanced by low zinc status. Specifically, absorption of zinc by carrier-mediated mechanisms is enhanced with low zinc status, suggesting that the total amount of zinc absorbed is homeostatically regulated. However, how zinc status regulates absorption of the mineral is unclear.

Inhibitors of Zinc Absorption Many compounds in food may complex with zinc and inhibit its absorption. Inhibitors include:

- *Phytate*, also called phytic acid, inositol hexaphosphate, or inositol polyphosphate, is found in plant foods, particularly legumes and cereals such as maize and bran. It binds to zinc (as well as other minerals) using oxygen. The zinc-phytate complex is large, insoluble, and poorly absorbed. However, fermentation of bread reduces the phytate content and improves zinc absorption. Figure 12.10 depicts the binding of zinc by phytate.
- *Oxalate* or oxalic acid, another inhibitor of zinc absorption, is found in a variety of foods, most notably spinach,



- ① Zinc is mostly absorbed (following release by proteases and nucleases) by Zrt- and Irt-like protein (ZIP4) into the enterocyte.
- ② Within the intestinal cell, zinc may be used functionally, stored as part of metallothionein, or attached to CRIP and transported to the basolateral membrane.
- ③ Zinc is transported across the basolateral membrane out of the intestinal cell by ZnT1. In portal blood, zinc binds mostly to albumin for transport to the liver.

Figure 12.9 Digestion, absorption, enterocyte use, and transport of zinc.

chard, berries, chocolate, and tea. The binding of zinc by oxalate is shown in Figure 12.10.

- *Polyphenols* such as tannins in tea and certain *fibers* found in whole grains, fruits, and vegetables also bind zinc and inhibit its absorption.
- *Nutrients* (vitamins and divalent cations) that affect zinc. Interactions between zinc and nutrients such as the vitamin folate and a variety of divalent cations (Fe^{2+} , Ca^{2+} , Cu^{2+}) may occur and inhibit zinc absorption. The results of studies examining folate's inhibition of zinc absorption (with folic acid given in amounts of 350 to 800 μg and zinc in amounts of 3.5 to 50 mg) are equivocal. However, given the use of folic acid supplements to prevent neural tube defects in women during childbearing years, additional studies are warranted.

The interaction between zinc and other divalent cations is thought to be related to the fact that various cations compete with one another for binding ligands in the

intestinal lumen or within the cell as well as for transporters on the brush border of the enterocytes [5,7,8]. Iron (nonheme) and zinc interact primarily when coingested in solution; the effects are not always apparent when given

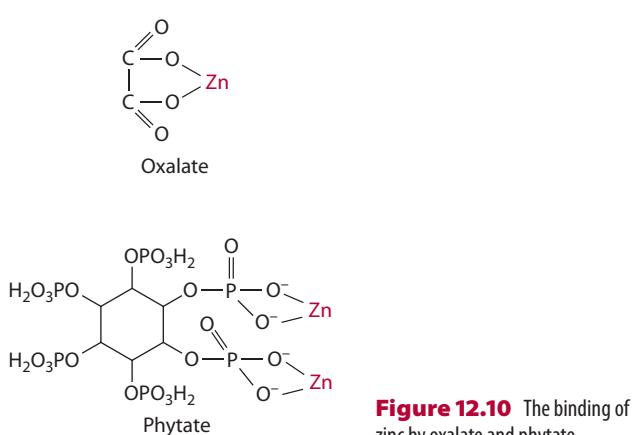


Figure 12.10 The binding of zinc by oxalate and phytate.

with a meal [7]. Zinc absorption is most commonly inhibited when the amount of the nonheme iron exceeds that of zinc and is given in amounts of 20 mg or higher. For example, ferrous sulfate and zinc sulfate ingested together in a ratio of 2:1 (50 mg:25 mg) and 3:1 (75 mg:25 mg) decreased zinc absorption [8]. Heme iron does not have the same effects on zinc, nor does ingestion of nonheme iron with zinc from food [8]. These studies suggest that to maximize zinc absorption, the zinc supplement should not be consumed with nonheme iron supplements.

The effects of calcium on zinc absorption and balance appear to vary. Some studies have shown that ingesting from 500 mg to ~2 g of calcium as calcium carbonate, hydroxyapatite, or calcium citrate malate has no effect on zinc absorption, whereas other studies providing similar amounts of calcium as milk, calcium phosphate, and calcium carbonate found a reduction in net zinc absorption and zinc balance [9–11]. Results appear to vary with the forms and amounts of the nutrients and the population used in the study.

Although copper has the potential to interfere with zinc absorption, such interference has not been reported. In fact, the opposite appears to occur; that is, zinc supplements inhibit copper absorption and can lead to copper deficiency (see the section “Interactions with Other Nutrients”).

Intestinal Cell Zinc Use

Movement of zinc through the enterocyte is not a well delineated process. As indicated in Figure 12.9, zinc entering the enterocyte has several possible fates. The zinc may be:

- used functionally within the enterocyte
- stored in the enterocyte
- transported through the cell and across the basolateral membrane into the plasma for use by other tissues

The use of zinc within the enterocyte is similar to its use in other body cells and is described further in the section “Functions and Mechanisms of Action.” Two proteins, cysteine-rich intestinal protein (CRIP) and metallothionein, serve as intracellular binding ligands for zinc. Once in the enterocyte, zinc initially appears to accumulate on CRIP. However, with increased zinc concentrations, zinc binds more to the protein thionein. Thionein contains an unusually high content of cysteine (30% cysteine residues), which functions in metal binding. Once the zinc is attached to thionein, the protein is called metallothionein. The zinc captured and held bound to metallothionein in the enterocytes is typically lost into the lumen with the sloughing of these cells, unless the metallothionein is degraded before cell turnover. Metallothionein is covered in further detail in the section on storage. Zinc not stored as metallothionein may be transported into cytosolic vesicles by a variety of zinc transporters, especially ZnTs and ZIPs.

Zinc not bound to metallothionein or used within the enterocyte may be transported across the basolateral

membrane of the enterocyte and into the blood for transport to other tissues. Several zinc transporters (ZnTs) have been identified. ZnT-1 is found in many tissues throughout the body, including the enterocyte basolateral membrane. Generally, ZnT proteins mediate zinc efflux from cells or movement of zinc into intracellular compartments, to lower cytosolic zinc concentrations [12]. ZnT-1 preferentially transports zinc out of the duodenal and jejunal intestinal cells and does not require sodium or ATP [13]. Synthesis of the protein is increased with high dietary zinc intake, but does not appear to be affected with decreased zinc intake [4]. In addition to ZnTs, a sodium-zinc exchanger has been identified that appears to affect sodium-dependent active extrusion of zinc from some cells [14].

Other zinc transporters, such as ZnT-2, ZnT-3, and ZnT-4, have been isolated in the intestine, kidney, testes, mammary glands, and brain, for example [13]. These transporters may be involved in intracellular zinc movement. In addition, ZIP carriers, which generally transport zinc into cells from extracellular locations, also transport zinc out of intracellular compartments and into the cytosol to effect an increase in cytosolic zinc concentrations [12]. Not all ZIP carriers, however, solely transport zinc. Some ZIP transporters (1, 2 and 3) carry other minerals too. The transporter ZIP5 is expressed in the intestinal cell as well as the pancreas, liver, and kidney. In the intestine, ZIP5 is found in the basolateral membrane, where it is thought to facilitate serosal to mucosal zinc transport [15]. In other words, ZIP5 moves zinc out of the body, that is, from the blood into the intestinal cell and then into the lumen of the gastrointestinal tract for excretion.

Transport

Zinc passing into portal blood from the intestinal cell is mainly transported loosely bound to albumin. Most zinc is then taken to the liver, where the mineral is initially concentrated. Zinc leaving the liver is transported in the blood still bound to albumin, but also may be attached to transferrin, α -2 macroglobulin, and immunoglobulin (Ig) G. Albumin is thought to transport up to ~60% of zinc in the blood. Transferrin, α -2 macroglobulin, and immunoglobulin (Ig) G are thought to transport ~15% to 40% of the zinc in the blood (Figure 12.11). Two amino acids, histidine and cysteine, loosely bind anywhere from <1% to about 8% of the zinc for transport; these amino acids form a ternary (histidine-zinc-cysteine) complex in the blood. Normal plasma zinc concentrations range from about 80 to 120 $\mu\text{g}/\text{dL}$ (12–18 micromol/L).

Uptake by Tissues

Multiple transport systems have been identified to facilitate cellular zinc uptake, yet the exact mechanisms are still unclear. ZIP carriers 1, 2, 4, 6, 7, 8, and 14 appear to be involved in cellular zinc uptake or the release of zinc from intracellular stores [4]. ZIP14, for example, transports zinc

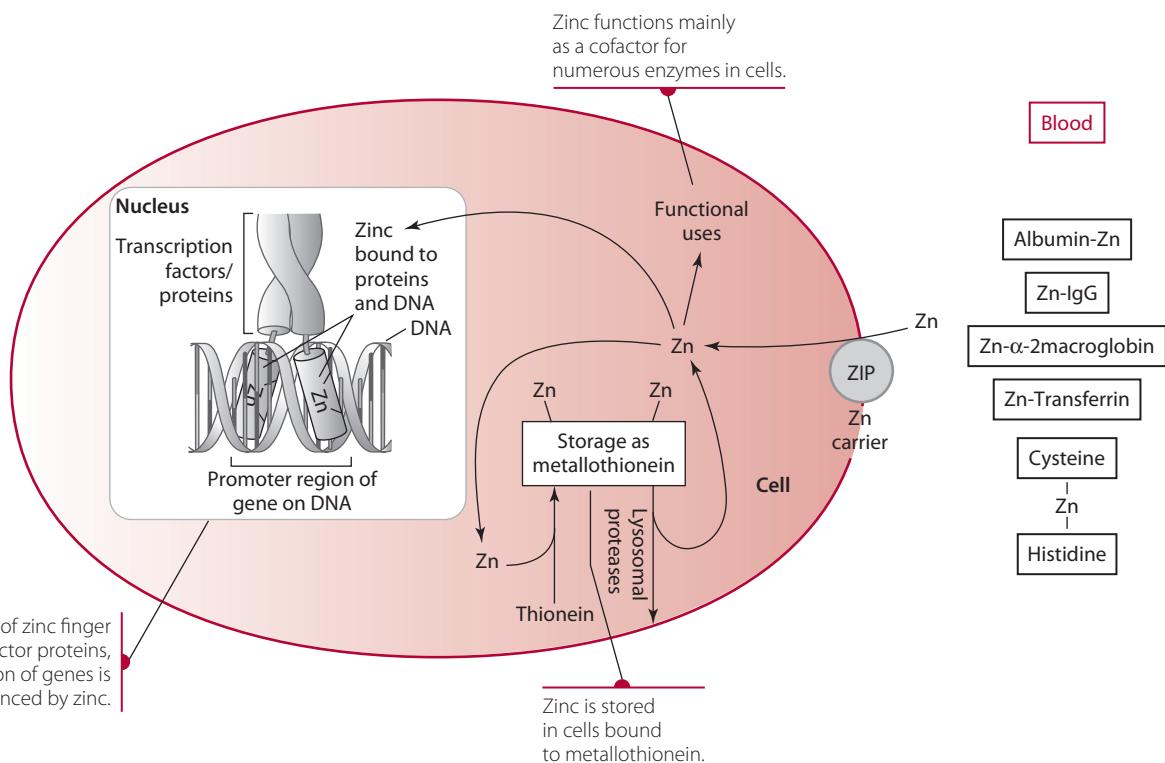


Figure 12.11 Uptake of zinc into cells, storage of zinc, and use as zinc-finger transcription factor protein to affect gene transcription.

into hepatocytes, and its activity appears to be increased as part of the acute phase (reactant) response (as occurs with infections or trauma). Thus, for example, with infections, zinc is preferentially taken up by the liver and stored there. This sequestering of zinc in the liver creates low blood zinc concentrations (hypozincemia) and prevents microorganisms in the blood from using the zinc for its own multiplication and growth. ZnT transporters also have a variety of roles in zinc efflux or in intracellular sequestration of zinc in cellular organelles or vesicles [4]. Some zinc carriers are not thought to be highly selective, but others are tissue-specific.

An increase in amino acid use by tissues leads to increased uptake of zinc, and vice versa. Given that numerous metalloenzymes within the cell require zinc as a component, it is reasonable to assume that enzyme synthesis and zinc uptake are correlated.

Distribution and Storage

Zinc is found in all body organs, most notably the liver, kidneys, muscle, skin, and bones. The zinc content of most soft tissues (including muscle, brain, lung, and heart) is relatively stable. This soft-tissue zinc does not respond to or equilibrate with other zinc pools to release zinc when dietary zinc intake is low. Furthermore, although zinc is found in bones as part of apatite, bones release the mineral very slowly and cannot be depended on to supply zinc during dietary deprivation. Instead, when dietary zinc intake is insufficient to meet the body's needs, plasma zinc-containing enzymes and metallothionein provide zinc. Catabolism of selected "less essential" zinc-containing metalloproteins (enzymes)

and liver metallothionein occurs so that zinc can be redistributed to meet particularly crucial needs for the mineral.

Zinc is thought to be stored in most tissues as part of the protein thionein, which when mineral bound is known as metallothionein. Metallothionein is found in most tissues of the body, including the liver, pancreas, kidney, intestine, and red blood cell. Various forms of the protein appear to exist. These forms are designated by number as metallothionein (MT)-1 through MT-4. MT-1 and MT-2 appear to be the most common tissue forms. As discussed in the section "Intestinal Cell Zinc Use," metallothionein contains a high proportion of cysteine residues (20 of 61 amino acids), each of which binds metals. In addition to binding zinc (7 g atoms/molecule), metallothionein binds copper, cadmium, and mercury.

Although metallothionein is thought to serve as a storage form of zinc, other roles also have been attributed to the protein. Metallothionein may serve as a zinc chaperone and transfer the mineral to acceptor proteins such as aconitase in the mitochondria [16]. Synthesis of the protein increases in times of stress; metallothionein is known as an acute phase (reactant) protein. Moreover, metallothionein exhibits radical scavenging antioxidant-type functions. For example, the protein is known to scavenge hydroxyl radicals.

Liver and red blood cell metallothionein concentrations diminish as dietary zinc intake decreases and thus is thought to reflect zinc status or stores. Zinc, and possibly other minerals, appears to affect the gene expression of thionein. Specifically, metal regulatory elements (MREs) made of specific nucleotide sequences are found in the

promoter region of the thionein gene. Zinc may interact with the MREs alone or through a metal transcription factor (abbreviated MTF) to induce thionein synthesis. Thionein gene expression is also influenced by glucagon and interleukin 1. Interleukin 1, synthesized and secreted by monocytes and activated macrophages, is thought to induce thionein gene transcription during infection and help promote the storage of zinc bound to metallothionein in the liver during infections [17].

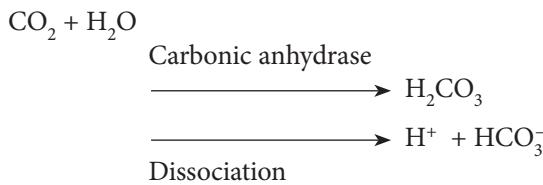
Release of zinc from metallothionein involves lysosomal proteases (Figure 12.11). At an acid pH, these proteases degrade metallothionein to release the zinc, which is then available for use by cells or other tissues.

FUNCTIONS AND MECHANISMS OF ACTION

Zinc has many seemingly divergent functions, probably because it is a component of numerous metalloenzymes. As a component of metalloenzymes, zinc provides structural integrity to the enzyme by binding directly to amino acid residues, participates in the reaction at the catalytic site, or both. Zinc appears to be part of more enzyme systems than all the rest of the trace minerals combined. Zinc affects many fundamental life processes (Table 12.4). Enzymes (at least 70 and perhaps over 200) from every enzyme class require zinc. A few of these zinc-dependent enzymes are described in the next section.

Zinc-Dependent Enzymes

- *Carbonic anhydrase*, found primarily in the erythrocytes and in the renal tubule, is essential for respiration. It catalyzes the following reaction, thereby allowing rapid disposal of carbon dioxide:



The H⁺ dissociated from carbonic acid reduces oxyhemoglobin as oxygen is released to the tissues; the bicarbonate

Table 12.4 Selected Functions of Zinc

Metalloenzyme component
Oxidoreductase
Hydrolase
Lyase
Isomerase
Transferase
Ligase
Gene expression
Cell replication
Membrane and cytoskeletal stabilization
Structural role in hormones

passes into the plasma to participate in buffering reactions. The amount of zinc associated with carbonic anhydrase and carried by the erythrocytes is approximately eight to nine times as much as that distributed to tissues in plasma [18]. Carbonic anhydrase has a very high affinity for the mineral zinc, which plays a catalytic role. Catabolism of this enzyme apparently does not occur even with zinc deprivation, but activity in red blood cells diminishes with low-zinc (3.8 mg for several weeks) diets [19].

- *Alkaline phosphatase* contains four zinc atoms per enzyme molecule. Two of the four ions are required for enzyme activity. The other two are needed for structural purposes. The enzyme, found mainly in bone and in the liver (with small amounts in the plasma), lacks substrate specificity, hydrolyzing monoesters of phosphates from various compounds. Enzyme activity decreases with zinc deficiency.
- *Alcohol dehydrogenase* also contains four zinc ions per enzyme molecule, with two of the four required for catalytic activity and two required for structural purposes (protein conformation). This enzyme is important in the conversion of alcohols to aldehydes (e.g., retinol to retinal, which is needed for the visual cycle and night vision). NADH also participates in the reaction.
- *Carboxypeptidase A* (Figure 12.12), an exopeptidase secreted by the pancreas into the duodenum, is necessary

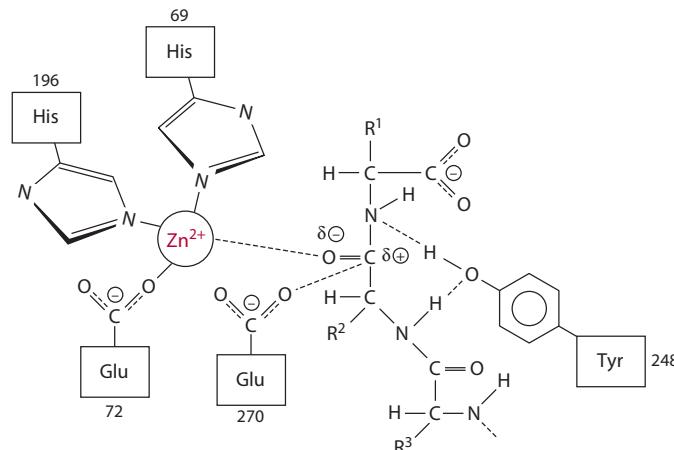
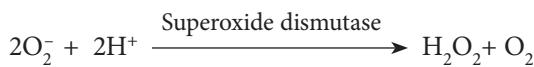


Figure 12.12 Partial structure of carboxypeptidase A.

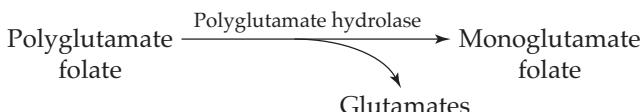
to digest protein. Zinc is bound tightly to carboxypeptidase A and is essential for enzymatic activity. Carboxypeptidase A activity decreases with zinc deficiency.

- *Aminopeptidase* is also involved in protein digestion. Aminopeptidases contain one zinc atom, needed for catalytic activity. The enzyme cleaves amino acids from the amino terminal end of the protein or polypeptide that is being digested in the intestinal tract.
- *Δ-aminolevulinic acid dehydratase*, involved in heme synthesis, is also zinc-dependent. This thiol (SH)-containing enzyme is made up of eight subunits, each of which binds one zinc atom. Zinc is essential for the maintenance of free thiols in the enzyme. The enzyme catalyzes the condensation of two Δ -aminolevulinic acids to form porphobilinogen (Figure 12.6). Lead, if present in the body in high concentrations (as occurs with lead poisoning), replaces zinc in the dehydratase and diminishes heme synthesis.
- *Superoxide dismutase (SOD)* found in the cell cytoplasm requires two atoms each of zinc and copper for function; zinc appears to have a structural role in the enzyme. An extracellular form of the enzyme that is also zinc- and copper-dependent has been characterized and appears to be more sensitive to zinc than is the cytosolic form of the enzyme. The extracellular form is found in the plasma, lymph, synovial fluid, and lungs; it exists in equilibrium between cell surfaces and the plasma. Both the cytosolic and extracellular forms of SOD serve important antioxidant defense roles in the body by catalyzing the removal of superoxide radicals, O_2^- .



Further information on SOD is found in the section on copper, and in the Chapter 10 Perspective.

- *Collagenases* help to digest collagen in the gastrointestinal tract. Zinc plays a catalytic role in the enzyme's function.
- *Phospholipase C* requires three zinc atoms for catalytic activity. This enzyme hydrolyzes the glycerophosphate bond in phospholipids.
- *Polyglutamate hydrolase*, also called γ -glutamylhydrolase or pteroylglutamate hydrolase, is a zinc-dependent enzyme necessary to digest folate in the gastrointestinal tract. Folate is found in foods bound to several (poly)glutamate residues. For folate to be absorbed, most of the glutamate residues must be removed.



Polyglutamate hydrolase catalyzes the hydrolysis of the glutamate residues from folate to yield monoglutamate folate, which is then actively transported into

the intestinal cells. Thus, poor zinc intake or status can diminish folate absorption.

■ *Polymerases, kinases, nucleases, transferases, phosphorylases*, and *transcriptases* all require zinc. Paramount in nucleic acid synthesis are the zinc metalloenzymes DNA and RNA polymerase and deoxythymidine kinase. Deoxythymidine kinase is necessary for the conservation or salvaging of thymine, the pyrimidine unique to DNA. Catabolism of RNA appears to be regulated by zinc because of zinc's influence on ribonuclease activity. Enzymes such as deoxynucleotidyl transferase, nucleoside phosphorylase, and reverse transcriptase also depend on zinc.

Other Roles

Physiological functions of zinc include tissue or cell growth, cell replication, bone formation, skin integrity, cell-mediated immunity, and generalized host defense. The role of zinc in tissue growth is related primarily to its function in regulating protein synthesis, which includes its influence on polysome conformation as well as the synthesis and catabolism of the nucleic acids.

With respect to transcription, zinc appears to interact with nuclear proteins (also called transcription factors or DNA-binding proteins) that bind to promoter sequences of specific genes on DNA (Figure 12.11). Thus, zinc helps regulate transcription. Specifically, zinc serves as a necessary structural component of DNA-binding proteins/transcription factors that contain zinc fingers. In fact, about 2,000 transcription factors appear to require zinc for structural integrity. *Zinc fingers* is a term used to indicate the shape (configuration) of the proteins, which look like fingers, and the presence of the mineral zinc bound to the protein. The finger-like configuration results from the twisting and coiling of the cysteine and histidine residues to which zinc binds in that segment of the protein. DNA-binding proteins that contain zinc fingers also bind other substances, such as retinoic acid, thyroxine, $1,25\text{-}(OH)_2$ vitamin D, and other steroid hormones such as estrogen and androgens. Thus, hormones such as retinoic acid or $1,25\text{-}(OH)_2$ vitamin D enter the cell nucleus and bind to specific protein-containing zinc fingers. In the presence of zinc, which is required for the binding of the protein to the DNA, the protein (with the hormone attached to it) binds to the DNA to affect gene expression.

The effect of zinc on cell membranes may occur through direct effects on the membrane proteins' conformation or on protein-to-protein interactions [20]. Zinc may affect the activity of several enzymes attached to plasma membranes, including alkaline phosphatase, carbonic anhydrase, and superoxide dismutase, among others [20]. Zinc itself also is believed to stabilize membrane structure by stabilizing phospholipids and thiol (SH) groups that need

to be maintained in a reduced state [21]. Zinc may also stabilize membranes by quenching free radicals as part of metallothionein and by promoting associations between membrane skeletal and cytoskeletal proteins [20]. Zinc in cells is found bound to tubulin, a protein that makes up the microtubules. Microtubules are thought to act as a framework for structural support of the cell as well as being needed for movement.

Zinc influences carbohydrate metabolism. Specifically, zinc is associated with insulin within stored granules in pancreatic beta cells. Further, ZnT5 is found in pancreatic beta cells and is associated with insulin-secreting granules [4]. Zinc deficiency decreases insulin response, resulting in impaired glucose tolerance. Zinc also appears to regulate the mammalian target of rapamycin (mTor), thereby affecting insulin-signalling and protein-synthesis pathways. Zinc also appears to influence the basal metabolic rate (BMR); a decrease in thyroid hormones and BMR has been observed in subjects receiving a zinc-restricted diet [22]. Zinc is also important for taste; it is a component of gustin, a protein involved in taste acuity. Zinc is important for cell survival. Apoptosis (programmed cell death) especially among some cells such as pre T- and B-cells, has been shown to be associated with zinc deficiency [23].

Finally, zinc is involved in host defense. Zinc deficiency affects both cell-mediated and humoral immunity. The literature in this area is extensive, but one example of the effects of zinc is through its actions on thymulin, a zinc-dependent hormone peptide that binds to T-cells and promotes their differentiation and functions (including cytokine release). T-cells are critical to immune system function and with zinc deficiency, thymulin activity diminishes and profoundly affects T-cell numbers and functions. The relationship between immunity and zinc has led many to use zinc lozenges to self-treat colds.

Zinc lozenges are purported to assist in the treatment of colds and infections. However, a meta-analysis of zinc salt lozenges and colds found no statistically significant benefit associated with the use of zinc lozenges for the treatment of colds [24]. More recent studies continue to provide conflicting results [25–28]. Thus, further study appears to be needed.

Although many functions of zinc are known, many of its roles are not known. The effects of zinc deficiency on the body fail to explain fully the manifestations of zinc deprivation. It is “the small fraction of total body zinc that exchanges relatively rapidly with plasma zinc that is responsible for many of the known physiological functions of zinc” [29].

INTERACTIONS WITH OTHER NUTRIENTS

Some of the interactions that inhibit zinc absorption have been addressed in the section on absorption. Interactions

between zinc and nutrients that do not affect zinc absorption are briefly covered here.

Zinc and vitamin A interact in several ways. From the discussion on zinc functions, you may remember that zinc is required for alcohol dehydrogenase. Retinol (vitamin A) serves as a substrate for this enzyme, which converts retinol to retinal (retinaldehyde). In addition, zinc is necessary for the hepatic synthesis of retinol-binding protein, which transports vitamin A in the blood [30]. Zinc deficiency is associated with decreased mobilization of retinol from the liver (even with adequate liver vitamin A stores) as well as decreased concentrations of several transport proteins found in the blood, including albumin, transferrin, and prealbumin [30].

The detrimental effect of excessive zinc intake on copper absorption is thought to be attributable to zinc's stimulation of thionein synthesis. Thionein polypeptides have a higher affinity for copper than for zinc. Thus, if zinc intake is high or increasing significantly, it causes increased synthesis of thionein. Any copper ingested in foods easily becomes trapped as part of metallothionein within the enterocyte [31,32]. The formation of copper metallothionein traps the copper in the enterocyte, preventing its passage into the plasma. The danger of copper deficiency precipitated by zinc supplementation has led to the recommendation of a tolerable upper intake level of elemental zinc of 40 mg daily [33].

Diminished calcium absorption has been observed with ingestion of zinc supplements when calcium intake is low (<300 mg calcium). However, calcium absorption appears to be unaffected by zinc when calcium intake is at adequate (recommended) levels.

Cadmium appears to bind to sites to which zinc would normally bind and to disrupt normal zinc functions. For example, cadmium can replace zinc in zinc fingers, causing the fingers to no longer function as they would with zinc present.

EXCRETION

The three primary routes of zinc loss from the body are through the:

- gastrointestinal tract
- kidneys
- skin (integument and sweat)

Most zinc is lost from the body through the gastrointestinal tract in the feces. Endogenous zinc in the form of enzyme metalloproteins is secreted by the salivary glands, intestinal mucosa, pancreas (main source), and liver into the gastrointestinal tract. Although some of this zinc is reabsorbed, some also is excreted in the feces. Zinc is also contributed to the gastrointestinal lumen by sloughed intestinal cells and possibly by enterocytes that may permit a bidirectional flow of the mineral.

In contrast to intestinal zinc losses, renal and dermal losses of zinc, as well as zinc losses in semen and menses, are relatively constant. Most zinc filtered by the kidneys is reabsorbed by the tubules. ZnT1 is thought to be involved with resorption of zinc in the kidney. Thus, only a small amount of zinc (~0.3–0.7 mg/day) is excreted in the urine. The zinc appearing in the urine is believed to be derived from the small percentage of plasma zinc that is complexed with histidine and cysteine. Zinc losses of ~0.4 to 0.6 mg/day occur with exfoliation of skin and with sweating. Other minor routes of zinc loss include (for men) semen (0.1 mg/day) and (for women) menses (0.1 mg/day). Hair contains ~0.1 to 0.2 mg zinc/g hair.

RECOMMENDED DIETARY ALLOWANCE

An allowance for zinc appeared for the first time in the 1974 RDAs. The subcommittees on the 1989 and 2001 editions of the RDAs based zinc recommendations on the intake needed to maintain balance as well as on estimates of zinc absorption and body losses. Total zinc losses for adult men and women were calculated at 3.84 mg and 3.3 mg, respectively [33]. Zinc losses for men consisted of 0.63 mg urinary zinc, 0.54 mg integumental and sweat zinc, 0.1 mg semen zinc, and 2.57 mg endogenous intestinal zinc; for women, urinary zinc losses were 0.44 mg, integumental and sweat zinc losses were 0.46 mg, menses zinc losses were 0.1 mg, and endogenous intestinal zinc losses were 2.3 mg [33]. To account for absorption, the daily requirements for zinc for adult men and women were set at 9.4 mg and 6.8 mg, respectively, and the RDAs were set at 11 mg and 8 mg, respectively. These recommendations are lower than those set in 1989, which were 15 mg/day for adult men and 12 mg/day for adult women [34]. The 2001 RDA for zinc during pregnancy is 11 mg/day to cover the calculated need for growth of the fetus and placenta [33]. The zinc recommendation for lactating women is 12 mg/day [33]. The inside cover of the book provides additional RDAs for zinc for other age groups.

DEFICIENCY

Some population groups, especially the elderly and vegetarians, have been found to consume less than adequate amounts of zinc [35]. Conditions associated with an increased need for intake include alcoholism, chronic illness, stress, trauma, surgery, and malabsorption.

Signs and symptoms of zinc deficiency are growth retardation (an early response to zinc deficiency in children caused by inadequate cell division needed for growth), skeletal abnormalities from impaired development of epiphyseal cartilage, defective collagen synthesis or cross-linking, poor wound healing, dermatitis (especially around body orifices), delayed sexual maturation in children, hypogeusia (blunting of sense of taste), alopecia (hair loss), impaired immune function, and impaired protein synthesis.

SUPPLEMENTS

Zinc is found in many forms in supplements (oral tablets, lozenges, and sprays), including zinc oxide, zinc sulfate, zinc acetate, zinc chloride, and zinc gluconate. Each of the forms differs in the amount of zinc provided and in absorption. Zinc gluconate, for example, is approximately 14.3% zinc, whereas zinc sulfate is 23% zinc, and zinc chloride is 48% zinc. Zinc chloride and zinc sulfate are very soluble, as is zinc acetate. In contrast, zinc carbonate and zinc oxide are fairly insoluble. A comparison of zinc preparations suggested that zinc acetate was one of the best-tolerated zinc preparations when compared with zinc sulfate, zinc aminoate, zinc methionine, and zinc oxide; zinc oxide was least absorbed [36]. Zinc supplements should be consumed on an empty stomach, without simultaneously ingesting other mineral supplements. Gastric irritation is a common side effect.

TOXICITY

Excessive intake of zinc can cause toxicity. An acute toxicity with 1 to 2 g zinc sulfate (225–450 mg zinc) can produce a metallic taste, nausea, vomiting, epigastric pain, abdominal cramps, and bloody diarrhea [34]. Chronically ingesting zinc in amounts of about 40 mg (lower for some people) results in a copper deficiency [33,34]. The tolerable upper intake level for zinc has been set at 40 mg daily based on this interaction with copper [33].

ASSESSMENT OF NUTRITION

Evaluating zinc nutrition is difficult, owing to homeostatic control of body zinc. A variety of static indices have been used to assess zinc status, including measurements of zinc in red blood cells, leukocytes, neutrophils, and plasma or serum. The most common basis for assessment is serum or plasma zinc, with fasting concentrations about <70 µg/dL suggesting deficiency [37]. Zinc (fasting) in the plasma decreases only when the dietary intake is so low that homeostasis cannot be established without use of zinc from the exchangeable pool that includes plasma zinc [37]. Low fasting plasma zinc thus indicates that little zinc is present in the exchangeable zinc pool and reflects a loss of zinc from bone and liver [38]. Plasma zinc levels must be interpreted with caution, because concentrations are influenced by many factors unrelated to zinc depletion, including meals, time of day (diurnal variation), stress, infection, and medications such as steroid therapy and oral contraceptive agents. Postprandial zinc concentrations have been found to be more sensitive to low dietary zinc intake than fasting plasma zinc concentrations [39].

Metallothionein also has been used to assess zinc status. Concentrations of metallothionein respond to changes in dietary zinc. Use of serum zinc and serum metallothionein

provide evidence of poor zinc status if both are low. Elevations in serum metallothionein coupled with low serum zinc, however, usually suggest an acute phase response, and in such conditions these indices are not reliable.

Urinary zinc and hair zinc have also been used to assess zinc status, but they are not valid indicators. Urinary zinc excretion remains fairly constant over a range of intakes and diminishes only with severe zinc deficiency. Low hair zinc may be associated with chronic intake of dietary zinc in suboptimal amounts; however, the concentration of zinc in hair depends not only on delivery of zinc to the root but also on the rate of hair growth, which is affected by other conditions (including protein status). Additional research and the development of standardized procedures (to eliminate contamination from, for example, shampoo, and confounding variables such as variations arising from hair color, sampling sites, etc.) are required before hair zinc may be useful for assessing zinc status.

Measurement of the activity of zinc-dependent enzymes also has been employed as an index of zinc status. Unfortunately, no enzyme is considered at present to be a valid and reliable indicator of zinc status. Studies using enzymes as indicators typically have measured carbonic anhydrase or alkaline phosphatase, which "hold" zinc less securely than other zinc metalloenzymes. Ideally, measurements of activity should be taken before and after zinc supplementation. An oral zinc tolerance test has been used to assess zinc absorption from different meals or supplements. This test typically involves ingestion of 25 or 50 mg zinc as zinc acetate with a test meal or supplement. Changes in plasma zinc concentration are assessed and compared in the same subjects following consumption of different test meals or supplements on different occasions under standardized conditions [40].

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Copper

The copper content of the human adult body is on the order of 50 to 150 mg. Copper is found in the body in either of two valence states, the cuprous state (Cu^{1+}) or cupric state (Cu^{2+}).

SOURCES

The copper content of food varies widely, reflecting the origin of the food and the conditions under which the food was produced, handled, and prepared for use. The richest sources of copper are organ meats and shellfish, as shown in Table 12.5. Plant food sources rich in copper include nuts, seeds, legumes, and dried fruits. Potatoes, whole grains, and cocoa also are good sources of copper. In the United States, the median intake of copper from foods by adults is calculated to be 1,000 to 1,600 $\mu\text{g}/\text{day}$ [1].

Endogenous sources of copper also may be found in the gastrointestinal tract. Copper is secreted daily into the gastrointestinal tract in digestive juices in relatively large amounts. For example, the copper contents of saliva and gastric juice are $\sim 400 \mu\text{g}$ and $1,000 \mu\text{g}$, respectively; pancreatic and duodenal juices may contain up to $1,300 \mu\text{g}$ and $2,200 \mu\text{g}$, respectively [2].

DIGESTION, ABSORPTION, TRANSPORT, UPTAKE, AND STORAGE

Digestion

Most copper, primarily as Cu^{2+} but some as Cu^{1+} , in foods is bound to organic components, especially amino acids that make up food proteins. Thus, digestion is needed to free the bound copper before absorption can occur. Gastric hydrochloric acid and pepsin facilitate the release of bound copper in the stomach. Additional proteolytic enzymes in the small intestine may hydrolyze proteins to further release copper. Copper digestion is shown in Figure 12.13.

Absorption

Although copper is absorbed throughout the small intestine, especially the duodenum, the stomach also appears to possess some absorptive capacity. This absorption may be attributable to the solubilizing effect of the acidic

Table 12.5 Copper Content of Selected Foods

Food/Food Group	Copper (mg/100g)	Food/Food Group	Copper (mg/100g)
Seafood		Meat and poultry	
Oysters	4.40	Liver	4.48
Crabmeat	0.64	Chicken	0.06
Shrimp	0.30	Beef, sirloin	0.15
Lobster	1.94	Pork	0.09
Eggs and dairy products		Legumes (cooked)	0.25
Eggs	0.02	Nuts and seeds	1.10–2.22
Milk	0.009	Fruits	
Cheeses	0.03	Fresh	0.04–1.11
Grains and cereals		Dried	0.19–0.34
Rice and pasta (cooked)	0.07–0.10	Vegetables	0.02–0.13
Bread white/wheat	0.13/0.29	Potatoes	0.20
		Other, cocoa	3.79

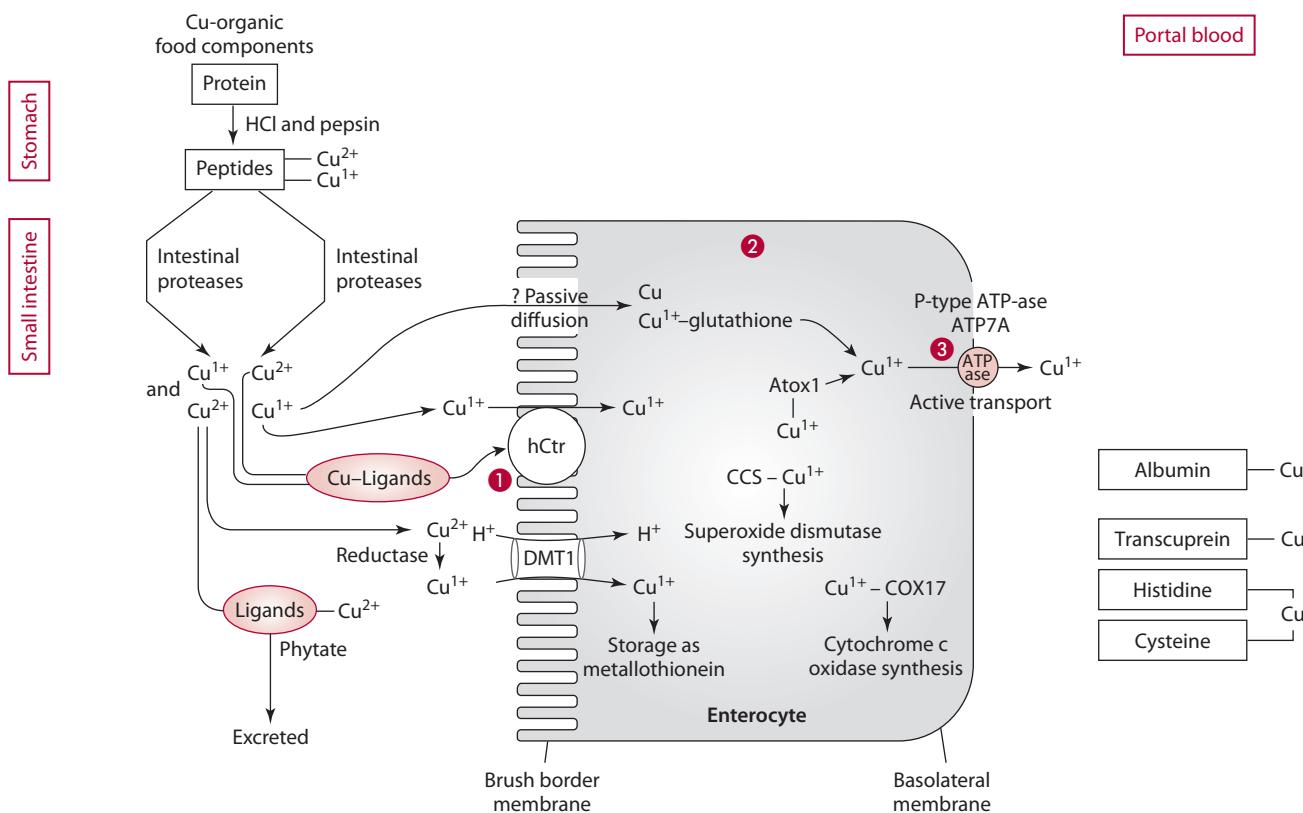
Source: www.nal.usda.gov/fnic/foodcomp.

environment on copper, which facilitates its transport across the gastric mucosa. However, compared to intestinal copper absorption, gastric copper absorption contributes relatively little to overall absorption.

The mechanisms for the absorption of copper across the brush border of the small intestine are not completely understood. It is possible that luminal copper must be bound to more absorbable ligands for effective transport. Even if copper is transported in the free ionic form, the presence of such ligands may be needed to present the metal to brush border receptors in a way that increases uptake.

Copper appears to be absorbed both by active carrier-mediated transporters and by a nonsaturable, passive diffusion process (Figure 12.13). As is true for other transport systems, low concentrations of dietary copper are transported primarily through the active carrier-mediated pathway, whereas the diffusion process accommodates higher concentrations. The major copper transporter is Ctr1. In fact, this transporter has been found in several body tissues, with the highest expression in the liver, heart, and pancreas and intermediate expression in the intestine [3–6]. Divalent mineral transporter 1 (DMT, also called divalent cation transporter—DCT—but hereafter designated as DMT) is also thought to transport Cu^{1+} to a limited extent. Several studies report competition between iron and copper for enterocyte brush border membrane absorption using DMT1 [7].

Most copper appears to be reduced by copper reductase activity on the brush border before being absorbed [8]. A cytochrome ferric/cupric reductase is thought to catalyze the reaction, which is stimulated by ascorbate. Specifically, ascorbate in the lumen of the gastrointestinal tract is thought to bind to the cytochrome reductase enzyme and reduce the copper to Cu^{1+} and then to complex with the reduced metal. The copper is then thought to be transported across the brush border membrane into the enterocyte by DMT1 in a $\text{Cu}^{1+}/\text{H}^+$ symport mechanism or by Ctr1 [8].



- ① Copper is mostly absorbed into the enterocyte by carriers such as hCtr and DMT1.
- ② Within the enterocyte, copper is chaperoned by various proteins such as glutathione, Atox1, CCS, and Cox17 which direct the copper for use within the cell.
- ③ Copper is transported out of the enterocyte by ATP7a.

Figure 12.13 Overview of copper digestion, absorption, enterocyte metabolism, and transport.

Typically, the gastrointestinal tract absorbs over 50% of ingested copper. However, the percentage of copper absorbed is influenced by copper status and dietary copper availability. Copper absorption is significantly higher during periods of low dietary copper than during periods of higher dietary copper [9]. Absorption, for example, may average only about 20% when copper intake is >5 mg/day but increases to over 50% when intake is <1 mg/day [2,7–9]. Copper absorption was calculated at 75%, based on intakes of ~350 µg [1,10].

Factors Influencing Copper Absorption

Copper transport across the brush border membrane may be influenced by a variety of dietary components, with some having a positive effect on absorption and others exerting a negative influence.

Enhancers of Copper Absorption Examples of substances that facilitate copper absorption include amino acids, especially histidine, as well as sulfur-containing amino acids such as methionine and cysteine. Whether copper bound to these amino acids can be absorbed through amino acid carrier systems is not clear. Copper also forms

ligands with amino acid sulphydryl groups in compounds such as glutathione.

Organic acids, other than vitamin C, in foods also improve copper absorption. Citric, gluconic, lactic, acetic, and malic acids act as binding ligands to improve solubilization of copper and thus absorption [11]. Citric acid forms a stable complex with copper and improves its absorption.

Inhibitors of Copper Absorption Many substances diminish copper absorption, including some substances in food.

- **Phytate** (inositol hexaphosphate or inositol polyphosphate; Figure 12.10), found mainly in plant foods (cereals, legumes), is a known inhibitor of copper (among other minerals, including iron, zinc, and calcium).

In addition to substances in foods, several trace minerals typically ingested in supplement forms are known to impede copper absorption.

- **Zinc** in amounts as low as 18.5 mg but typically in amounts of about 40 mg or more has been shown to impair copper absorption and diminish copper status [12–15]. The detrimental effect of excessive zinc intake

on copper absorption is thought to result from zinc's stimulation of thionein synthesis. Thionein, a cysteine-rich protein, however, more avidly binds copper than zinc. The copper bound to the thionein (now called metallothionein) ultimately is excreted as the intestinal cells are sloughed and the copper thus does not get into the body. If this problem occurs for an extended period of time, copper deficiency or suboptimal copper status can occur.

- **Iron** ingested in relatively large amounts decreases copper absorption in both rats and humans [16,17]. For example, copper absorption in infants fed formula supplemented with iron (10.8 mg iron/L) was significantly lower than that of infants fed a formula providing only 1.8 mg iron/L [17].
- **Molybdenum** as tetrathiomolybdate (MoS_4^{2+}) forms an insoluble complex with copper to inhibit its absorption in the gastrointestinal tract of rats and ruminants. The significance of these findings for humans is unknown.

Other nutrients also are known to impair copper absorption.

- **Calcium** and **phosphorus**, two major minerals, impair copper absorption. Calcium (2,382 mg as calcium gluconate) and phosphorus (2,442 mg as glycerol phosphate) were shown to increase fecal copper excretion compared with diets containing only moderate amounts of calcium (780 mg as calcium gluconate) with either high phosphorus (2,442 mg as glycerol phosphate) or moderate phosphorus (843 mg as glycerol phosphate) [18]. Urinary copper losses were also significantly greater on the high-calcium, high-phosphorus diet than on the moderate-calcium, moderate-phosphorus diet [18].
- Vitamin C may interact with copper to decrease its absorption. Presumably, vitamin C reduces copper from a cupric state (Cu^{2+}) to a less absorbable cuprous state (Cu^{1+}) [19,20]. Vitamin C has also been suggested to decrease copper retention.
- Excessive antacid ingestion or a high pH environment may diminish copper absorption and induce deficiency. Copper is absorbed best from a more acidic environment. Copper at a neutral or more alkaline pH binds to hydroxides (OH), forming insoluble compounds that are not readily absorbable.

Other factors also appear to influence copper absorption, including the body's copper status. The efficiency of absorption of copper changes to regulate in part whole-body copper status. Changes in fecal excretion also mediate the process. Thus, with high copper intake, less copper is absorbed. As body copper stores increase, the amount of copper excreted in the bile increases. Moderately low copper intake results in the reverse (increased absorption, decreased excretion) to some extent. However, for

example, with copper intake of ~0.38 mg/day, regulation does not sufficiently compensate to prevent depletion of body copper [21].

Intestinal Cell Copper Use

Once within the intestinal cell, copper may be stored, used by the cell, or carried through the cell's cytosol for subsequent transport across the basolateral membrane and into the blood for transport to tissues. Storage of copper occurs as part of metallothionein (see the section "Intestinal Cell Zinc Use" for a description of metallothionein). Unless released from storage, this copper is lost with intestinal cell turnover, approximately every 2 to 3 days. In addition to intestinal cell use and storage, some copper is transported across the enterocyte's cytosol for export across the basolateral membrane into the plasma.

Intracellular copper transport is not well characterized. Like iron, however, free copper ions may damage cells through nonenzymatic Fenton and Haber Weiss reactions (see the section "Other Roles"). Consequently, copper is found in the body typically bound to proteins. Amino acids and glutathione (a tripeptide composed of glycine, cysteine, and glutamate) are proposed copper (Cu^{1+}) carriers. Ctr2 transports copper into vesicles within the cell cytoplasm for temporary storage. In addition, chaperones (soluble intracellular proteins) bind intracellular copper and deliver it to various locations. Several chaperones have been identified, including cyclooxygenase (cox)17, atox1 (also called hAtx or Hah1), and CCS (copper chaperone for superoxide dismutase). Cox17, found in the cytosol, and cox 11, found in mitochondria are thought to transport Cu^{1+} for cytochrome c oxidase synthesis, whereas CCS (found in the mitochondria and cytosol) delivers Cu^{1+} for the synthesis of superoxide dismutase (SOD) from aposuper-oxide dismutase [3–6,22]. Atox1 ferries Cu^{1+} to cytosolic P (phosphorylation)-type ATPases necessary for cellular export of copper. Other possible chaperones include murr1 which is associated with ATP7B in the liver needed for copper excretion; APP found in selected cell membranes, and sco1 and sco2 found in the mitochondria [22].

Copper transport across the intestinal cell's basolateral membrane into the plasma is thought to occur primarily by active transport by a P-type ATPase called ATP7A [23,24]. Generally, ATPase transporters carry metals in their reduced state; however, whether this is true for copper transport is unclear. Whether ATP7A pumps the copper directly across the basolateral membrane or pumps it into a compartment, which then releases the copper by exocytosis, is not clear [25]. Mutations in the ATP7A gene are thought to result in Menkes' disease, an X-linked disorder characterized by defective copper transport (efflux), especially in the intestine and brain, where ATP7A typically functions. People with Menkes' disease have increased intestinal cell copper concentrations and impaired delivery of copper to peripheral

tissues. The condition is also characterized by vascular and neurological problems that are only partially alleviated by intravenous administration of copper.

Transport and Uptake

From the intestinal cell, copper is transported in portal blood to the liver bound loosely to the protein albumin. Specifically, the amino (N)-terminus of albumin has a high affinity for copper (Cu^{2+}). Copper also may be transported bound to transcuprein (Tc) and to amino acids such as histidine and cysteine [24].

The uptake of copper by the liver (and likely other tissues) is thought to occur by various carrier proteins such as those used in the enterocytes. In addition, transport across the cell membrane may involve the formation of certain amino acid–copper complexes and may also involve albumin.

Once within the liver, copper first appears to bind to metallothionein and then is slowly transferred to the copper enzymes, especially apoceruloplasmin. Six copper ions (in the form of Cu^{1+} and Cu^{2+}) are attached posttranslationally to apoceruloplasmin to form ceruloplasmin. Three of the six copper atoms are involved in electron transfer, and the other three function at the catalytic site, giving the protein a blue color. Although copper does not appear to influence apoceruloplasmin synthesis, ceruloplasmin activity is diminished or absent without sufficient copper, and ceruloplasmin's half-life is shortened.

Ceruloplasmin is released into the blood from the liver and constitutes about 60% (or perhaps up to 95%) of circulating copper in the blood after meals [11,26–28]. The remaining copper in the blood circulates loosely bound to albumin, transcuprein, and histidine [29].

Ceruloplasmin delivers copper to tissues. Uptake of ceruloplasmin copper by extrahepatic cells involves binding of ceruloplasmin to specific receptors [28]. The copper ions that are not at ceruloplasmin's active oxidase site are released [26]. Release is thought to involve reduction of copper from Cu^{2+} to Cu^{1+} . Ascorbic acid enhances copper transfer and is probably involved in the reduction of the copper [28]. Following dissociation from the ceruloplasmin, copper enters the cell either directly through channels or after binding to protein transporters such as hCtr1, 2, or 3.

Storage

Compared to other trace minerals, little copper ($<150 \text{ mg}$) is found in the body. Both the liver and the kidney rapidly extract copper from the blood. Other copper-containing tissues include the brain, heart, bone, muscle, skin, intestine, spleen, hair, and nails. Organs with the most copper per g include the liver, brain, and kidneys.

Within cells and tissues, copper is bound to amino acids, proteins, and chaperones. The liver appears to be the main storage site for copper, which is thought to be bound to metallothionein. Copper positively influences hepatic and renal, but not intestinal, thionein synthesis.

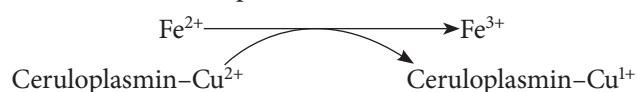
Metallothionein, in addition to storing up to about 12 copper atoms (as well as zinc atoms), protects cells by scavenging damaging superoxide and hydroxyl radicals. The amount of copper available to extrahepatic tissues is thought to be regulated by the liver through the synthesis of ceruloplasmin, through copper incorporation into metallothionein, and through excretion of copper into the bile.

FUNCTIONS AND MECHANISMS OF ACTION

The essentiality of copper is due, in part, to its participation as an enzyme cofactor and as an allosteric component of enzymes. Several copper-requiring metalloenzymes and the reactions they catalyze are described next. In many enzymes, copper functions as an intermediate in electron transfer.

Ceruloplasmin

Ceruloplasmin, a glycoprotein, is not simply a transporter of copper in the blood. It is also a multifaceted oxidative enzyme (oxidase) and antioxidant that may be found in the blood but also may be found bound to cell surface receptors on the plasma membranes of cells. Ceruloplasmin, also known as ferroxidase I, oxidizes minerals, most notably ferrous (Fe^{2+}) iron but also manganese (Mn^{2+}). Fe^{2+} must be oxidized to Fe^{3+} for iron to bind to transferrin so it can be transported to tissues.

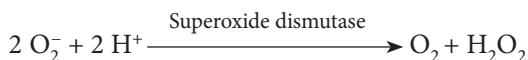


Another proposed function of ceruloplasmin is as a modulator of the inflammatory process and a scavenger of oxygen radicals to protect cells. As a modulator of the inflammatory process, many proteins, including ceruloplasmin, serve as acute phase (also called reactant) proteins. Acute phase proteins rise in the blood with, for example, infection or other inflammatory events (injury). This rise in the blood is important, because during infections phagocytosis of invading organisms by white blood cells generates superoxide radicals, among other damaging compounds. These compounds are normally generated, but they are generated in larger amounts with inflammation and must be eliminated (by ceruloplasmin, superoxide dismutase, or other enzymes) to prevent further damage to body cells.

Superoxide Dismutase

Superoxide dismutase (SOD), found both in the cytosol of cells and extracellularly, is copper- and zinc-dependent (another form in the mitochondria is manganese-dependent). In the enzyme, copper is thought to be linked with zinc through an imidazole group, and both minerals are linked to the enzyme protein by histidine and aspartate residues. Copper

(Cu^{2+}) is found at the enzyme's active site, where the superoxide substrate binds to the enzyme. Removal of copper, but not zinc, results in reduced cytosolic SOD activity. Specifically, superoxide dismutase catalyzes the removal (dismutation) of the superoxide radicals (O_2^-). During the reaction, copper is reduced along with the oxygen radical to initially generate molecular oxygen (O_2) and then, by reoxidation, hydrogen peroxide (H_2O_2).



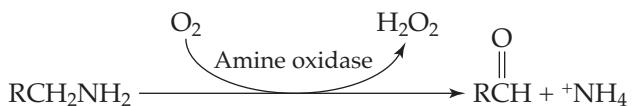
Superoxide radicals can cause peroxidative damage of phospholipid components of cell membranes. In other words, without SOD, superoxide radicals can form more destructive hydroxyl radicals that can damage unsaturated double bonds in cell membranes, fatty acids, and other molecules in cells (see the Perspective in Chapter 10). SOD therefore assumes a very important protective function. SOD is found in the cytosol of most cells of the body. Extracellular SOD is secreted and bound to heparan sulfate on the surface of cells and is found in relatively large concentrations in the arterial wall, where it may serve an important role in antioxidant defense (among other possible functions). Increased peroxidation of cell membranes is found with copper deficiency.

Cytochrome c Oxidase

Cytochrome c oxidase contains three copper atoms per molecule. One subunit of the enzyme contains two copper atoms and functions in electron transfer. The second subunit contains another copper atom involved in reducing molecular oxygen. Cytochrome c oxidase functions in the terminal oxidative step in mitochondrial electron transport (Figure 3.26). Specifically, the enzyme transfers an electron such that molecular oxygen (O_2) is reduced to form water molecules and enough free energy is generated to permit ATP production. Severe copper deficiency ultimately impairs the activity of this enzyme.

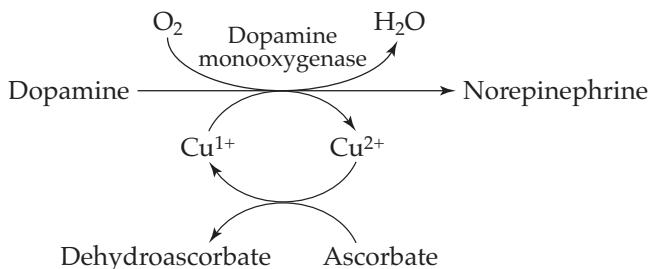
Amine Oxidases

Amine oxidases are also copper-dependent. Copper appears to function as an allosteric structural component of these enzymes, and TOPA quinone (6-hydroxydopa) serves as an organic cofactor [30]. Histidine residues in the enzyme serve as ligands for the copper. Amine oxidases, found both in the blood and in body tissues, catalyze the oxidation of biogenic amines such as tyramine, histamine, and dopamine to form aldehydes and ammonium ions (NH_4^+ is generated from the cleaved amine group). In the reaction, oxygen (O_2) is reduced to form hydrogen peroxide (H_2O_2). Other amino substrates include serotonin (5-hydroxytryptamine), norepinephrine, and polyamines.

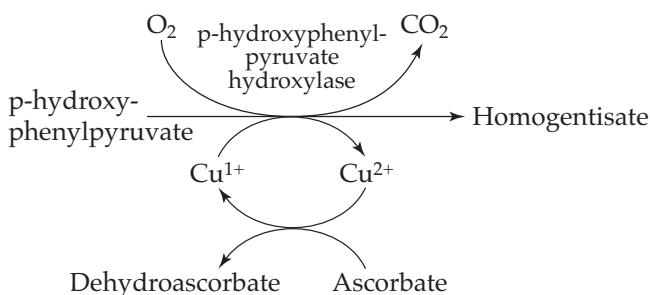


Tyrosine Metabolism—Dopamine Monooxygenase and p-hydroxyphenylpyruvate Hydroxylase

In tyrosine metabolism (Figure 6.28), norepinephrine and homogentisate production are both copper dependent. Norepinephrine synthesis begins with tyrosine, which is converted in an iron-dependent reaction to 3,4-dihydroxyphenylalanine (also called L-dopa). The L-dopa is further metabolized to dopamine. The enzyme dopamine monooxygenase converts dopamine to norepinephrine. This enzyme contains up to eight copper atoms per molecule and requires molecular oxygen and vitamin C for its function, as shown.



In tyrosine catabolism (Figure 6.28), the conversion of p-hydroxyphenylpyruvate to homogentisate also requires a copper-dependent hydroxylase and vitamin C, as shown.



Lysyl Oxidase

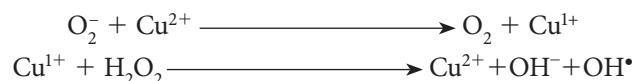
Lysyl oxidase, secreted by connective tissue cells (bone, blood vessels, etc.), generates cross-links between connective tissue proteins, including collagen and elastin. Specifically, lysyl oxidase catalyzes the removal of the ϵ amino group (oxidative deamination) of lysyl and hydroxylysyl residues of a collagen or elastin polypeptide and the oxidation of the terminal carbon atom of an aldehyde to form cross-links. The cross-linking is needed to stabilize the extracellular matrix. Lysyl oxidase activity decreases with inadequate copper intake, negatively affecting connective tissues [31].

Peptidylglycine α-amidating Monooxygenase

Amidation of peptide hormones, such as bombesin, calcitonin, gastrin, and cholecystokinin, is necessary for hormone function. The amidation requires a copper-dependent enzyme known as peptidylglycine α-amidating monooxygenase, which is found mostly in the brain. This enzyme cleaves a carboxy terminal glycine residue off peptides that have a C-terminal glycine. The amino group of glycine is retained by the peptide as a terminal amide. The oxidized residue is released as glyoxylate. Peptidylglycine α-amidating monooxygenase also requires vitamin C to reduce Cu²⁺ back to Cu¹⁺. This reaction is shown in Figure 9.5.

Other Roles

Copper plays a variety of other roles in the body that are not well understood; these roles may or may not involve enzymes. Some of these other roles include angiogenesis, immune system function, nerve myelination, and endorphin action. As a pro-oxidant, copper (if free) behaves similarly to iron. Copper (Cu²⁺) reacts with superoxide radicals and catalyzes the formation of hydroxyl radicals through the Fenton reaction:



Associated with the generation of reactive oxygen species is increased oxidative damage to DNA (base oxidation and strand breaks), proteins, and lipids (peroxidation), especially membrane lipids.

Copper influences gene expression through binding to specific transcription factors, also called binding proteins. In some cases, copper influences transcriptions by binding to transcription factors that in turn bind to promoter sequences on DNA. Once the Cu-bound transcription factors are bound to DNA, transcription may be enhanced or suppressed.

INTERACTIONS WITH OTHER NUTRIENTS

Copper is known to interact with a number of dietary constituents. Those that affect copper absorption have been described previously. Additional interactions are mentioned in this section.

Among organic dietary substances, ascorbic acid (1.5 g for 64 days) resulted in decreased serum ceruloplasmin activity; however, concentrations remained within the normal range [19]. An intake of 605 mg vitamin C for 3 weeks also resulted in a 21% decrease in serum ceruloplasmin oxidase activity [20]. The effects of vitamin C may be mediated through the reduction of the cupric ion to its cuprous form by the ascorbate, through the formation of a poorly absorbable complex, or by both mechanisms.

A strong, mutual antagonism exists between copper and zinc, most likely caused by the induction of intestinal

metallothionein by zinc. This results in excessive intracellular binding of the copper, reducing its luminal-to-serosal (e.g., from the lumen of the gastrointestinal tract across the basolateral membrane) flux, and entry into the blood. Zinc intakes ranging from 18.5 to 300 mg daily have resulted in copper deficiency [12–15]. Furthermore, copper deficiency induced by zinc intake (110–165 mg) for 10 months did not respond to cessation of zinc and 2 months of oral copper supplementation. Cupric chloride given intravenously for 5 days (total dose, 10 mg) was needed to correct the deficiency, suggesting that elimination of excess zinc by the body is a slow process and that zinc continues to inhibit copper absorption until it is entirely eliminated [14].

Another interaction of copper that has practical importance involves iron [32–34]. The importance of copper in normal iron metabolism is evidenced by the anemia that results from prolonged copper deficiency. The anemia is caused by impaired mobilization and use of iron, stemming from the reduced ferroxidase activity of hephaestin in intestinal enterocytes and of ceruloplasmin in the liver and plasma, which are responsible for oxidation of iron to its trivalent (Fe³⁺) state. Only as Fe³⁺ can iron efflux and bind to its transport protein, transferrin. With copper deficiency, the activity of ceruloplasmin and the expression of hephaestin are reduced. In contrast, high iron intake appears to interfere with copper mobilization from stores, copper use in the body, and copper absorption in infants and children [16,17,35,36].

As described in brief regarding factors inhibiting copper absorption, in animals (ruminants and rats) dietary copper forms insoluble complexes with molybdenum and sulfur in the form of tetrathiomolybdate (MoS₄)²⁺. Although such findings have not been reported in humans, urinary copper excretion in humans has been shown to rise from 24 to 77 µg/day as molybdenum intake increased from 160 to 1,540 µg/day [37]. No changes in fecal copper excretion were noted, suggesting that molybdenum may have increased copper mobilization from tissues and promoted excretion [37].

Copper and selenium also appear to interact. Copper deficiency has been shown to decrease the activity of the selenium-dependent enzymes glutathione peroxidase and 5'-deiodinase [38,39]. Antagonistic interactions of copper with cadmium, silver, and mercury have been reported but have more theoretical than practical importance.

EXCRETION

Copper is excreted primarily (>95%) through the bile, as reviewed by Wijmenga and Klomp [40]. Dietary copper intake directly influences biliary copper excretion such that low dietary copper intake results in low fecal copper excretion [21]. In other words, biliary copper excretion is regulated to maintain copper balance. At a copper intake of about 1.4 mg/day, endogenous fecal copper excretion is about 2.4 mg/day [24,41].

The P-type ATPase called ATP7B plays a major role in copper excretion. In the liver, ATP7B functions in the trans-Golgi network and cytoplasmic vesicles. ATP7B delivers copper in the trans-Golgi network for insertion into apoceruloplasmin or other apocuproenzymes (if cell copper is low), or it moves the copper to compartments (vesicles) for excretion in the bile (if cell copper is high). The copper-containing vesicles are thought to be exocytosed (in a process that involves murr1, a protein chaperone found in the liver) into hepatic caniculi for excretion into the bile. Wilson's disease, an inherited disorder of copper metabolism, is characterized by defective biliary copper excretion. Mutations in ATP7B are known to cause the disorder. Consequently, copper accumulates mainly in the liver but also in other organs, including the brain, kidney, eye (cornea), and spleen.

Only a small amount of copper ($<20\text{ }\mu\text{g}$) is excreted through the kidneys in the urine. Furthermore, urinary copper excretion does not change significantly with changes in copper intake except under extreme conditions [42]. Only small amounts ($<50\text{ }\mu\text{g}$) of copper are lost in sweat and with desquamation of skin cells. Women experience trace losses of copper in normal menstrual flow; however, a woman's copper status, unlike her iron status, is not compromised by menstruation. Trace amounts of copper also are lost with loss of hair and nails and, in males, in semen. Together, losses from menses or semen, along with losses from hair and nails, are not thought to exceed surface losses [1].

RECOMMENDED DIETARY ALLOWANCE

A recommendation for an estimated safe and adequate daily dietary intake for copper was first made in the 1980 RDAs. The 1989 estimated safe and adequate range for copper intake for adults was 1.5 to 3 mg/day [43]. Over the past decade, results of depletion and repletion studies, along with other studies permitting factorial analysis of obligatory losses over a range of intake, have enabled an estimate of copper requirements to be made. The requirement for copper for adult men and women was determined to be 700 μg [1]. The RDA for copper for adult men and women was set at 900 $\mu\text{g}/\text{day}$, based on a 30% coefficient of variation of the requirement and rounding to the nearest 100 μg [1]. Recommendations during pregnancy and lactation are 1,000 μg and 1,300 μg , respectively [1]. RDAs for copper for other age groups are found on the inside cover of the book.

DEFICIENCY

Various clinical manifestations are associated with copper deficiency. Recognized manifestations include hypochromic anemia, leukopenia (specifically neutropenia, a lower-than-normal number of neutrophils), hypopigmentation

or depigmentation of skin and hair, impaired immune function, bone abnormalities (especially demineralization), and cardiovascular and pulmonary dysfunction [43–46]. Changes in cholesterol concentrations have been reported in some but not all studies [47–49]. Because copper as ceruloplasmin is needed to oxidize iron (for mobilization from stores, export out of cells, and transport by transferrin), copper deficiency results in a secondary iron deficiency anemia. Treatment with copper (and not iron) is required to correct the problem.

The likelihood of copper deficiency increases in persons consuming excessive amounts of zinc (40 mg/day) or antacids as well as in persons with conditions that promote increased loss of copper from the body, as occurs with nephrosis or gastrointestinal malabsorptive disorders such as celiac disease, tropical sprue, and inflammatory bowel diseases.

TOXICITY

Copper toxicity (toxicosis) is fairly rare in the United States, although acute poisonings have occurred because of water contamination or accidental ingestion. A tolerable upper level for copper is set at 10 mg per day by the Food and Nutrition Board [1]. Copper intake of 64 mg (250 mg copper sulfate) has resulted in epigastric pain, nausea, vomiting, and diarrhea. Other symptoms of toxicity include hematuria (blood in urine), liver damage resulting in jaundice, and kidney damage resulting in oliguria (little urine production) or anuria (no urine production) [50]. Copper is lethal in amounts about 1,000 times normal dietary intake [51]. Chronic copper ingestion of 30 mg daily for 2.5 years followed by 60 mg copper daily for a year is reported to have resulted in liver failure in a young man who self-prescribed copper supplements [52].

Wilson's disease, a genetic disorder characterized by copper toxicity, results from mutation(s) in the gene coding for ATP7B [23]. The absence or dysfunction of ATP7B disrupts copper movement through the Golgi network into bile and into the secretory pathway for incorporation into selected proteins. In Wilson's disease, copper accumulates in organs, resulting in disturbed function of organs, especially the liver, kidneys, and brain. Kayser-Fleischer (greenish gold) rings caused by copper deposition also are visible in the cornea. At present, treatment of Wilson's disease involves avoiding high-copper foods and receiving D-penicillamine therapy to bind body copper and increase its excretion [53]. Zinc supplements ($>40\text{ mg}$ daily) along with tetrathiomolybdate also may be recommended to decrease copper absorption.

SUPPLEMENTS

The main form of copper used in mineral-fortified food products is copper sulfate; however, cupric oxide is still found in many vitamin-mineral supplements [54].

The use of cupric oxide as a source of copper is discouraged, because the copper has been shown to be unavailable for absorption from the gastrointestinal tract of animals; in fact, it is no longer used as a copper supplement in animal nutrition [54]. In addition to copper sulfate (~25% copper), other bioavailable and water-soluble forms of copper include cupric chloride (~47% copper), cupric acetate (~35% copper), and copper carbonate (~57% copper) [54].

ASSESSMENT OF NUTRITION

Copper status is best assessed using multiple indicators. Serum, plasma, or red blood cell copper is frequently used, but these indicators are likely inadequate to assess short-term changes in copper status. The lower end of the normal range for serum copper concentrations is reported to be 10 micromol/L. The change in plasma or serum copper concentration that occurs when subjects consume inadequate copper varies considerably between individuals and is further affected by several factors unrelated to diet. Extremely low copper intake (~0.38 mg/day), however, appears to be sufficient to significantly decrease not only plasma copper but also ceruloplasmin concentration and activity as well as urinary copper excretion [55]. Many other studies also have shown decreases in serum ceruloplasmin concentrations and activity with copper deficiency. The lower end of the normal range for serum ceruloplasmin is 180 mg/L, although levels <20 mg/L have been reported with copper deficiency [56,57]. The ratio of ceruloplasmin enzyme activity to protein concentration is thought to be better than either measurement alone [58,59].

Response of serum ceruloplasmin to copper supplements also may be used to assess copper status. Typically, supplemental copper first normalizes serum copper and neutrophil count, then serum ceruloplasmin [44]. Ceruloplasmin concentration increases following supplementation only in copper-deficient subjects. Another useful indicator of copper status is measurement of the activity of copper-dependent enzymes such as superoxide dismutase (SOD) (normal is 0.47–0.067 mg/g) in the red blood cell. SOD activity is sensitive to longer-term copper deficiency [56]. Platelet copper concentrations, along with platelet or leukocyte cytochrome c oxidase or skin lysyl oxidase activity, also have shown response to changes in copper status.

Copper concentrations in hair have not been correlated with either serum or organ copper, even though they are reduced with a prolonged period of copper deficiency. Hair concentrations therefore are not thought to be useful indicators of copper status, nor is urinary copper excretion, which normally is very low and is responsive to change only when intake is so low that other indicators have already declined [51].

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Selenium

Selenium, a nonmetal, exists in several oxidation states, including Se^{2-} , Se^{4+} , and Se^{6+} . The chemistry of selenium is similar to that of sulfur; consequently, selenium can often substitute for sulfur. The total body selenium content ranges from about 13 to 30 mg.

SOURCES

Perhaps more than any other essential trace element, selenium varies greatly in its soil concentration throughout the regions of the world. Consequently, the selenium content of plant foods and products is extremely variable. Cereals and grains may contain from less than 10 $\mu\text{g}/100\text{ g}$ to over 80 $\mu\text{g}/100\text{ g}$. Animal products (especially organ meats) typically contain from ~40 to 150 $\mu\text{g}/100\text{ g}$. Muscle meats generally provide between 10 and 40 μg selenium/100 g. Dairy products contain less than ~30 $\mu\text{g}/100\text{ g}$. Seafood is also thought to represent one of the better sources of selenium, although the bioavailability of selenium from fish (if the fish contains mercury) may be poor because of formation of unabsorbable mercury-selenium complexes [1].

ABSORPTION, TRANSPORT, UPTAKE, STORAGE, AND METABOLISM

Selenium occurs naturally in foods almost exclusively in the form of organic compounds, primarily selenomethionine and selenocysteine (Figure 12.14). These organic forms represent selenium analogues of sulfur-containing amino acids. The element substitution is made possible by the chemical similarity between selenium and sulfur. These selenium analogues become incorporated into plant proteins, which in turn may be eaten by animals.

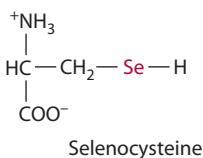
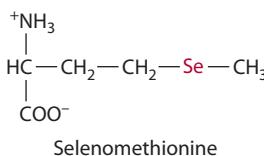


Figure 12.14
Selenomethionine and selenocysteine.

Selenomethionine tends to be found primarily in plant foods, whereas selenocysteine is found mostly in animal products.

Inorganic forms of selenium include selenide (H_2Se), selenite (H_2SeO_3), and selenate (H_2SeO_4). These inorganic forms are found in various vegetables (such as beets and cabbage) as well as in yeast. In addition, in parts of the world where selenium levels in natural foodstuffs are low, animal feeds generally are supplemented with sodium selenite. Supplements generally provide selenium as selenomethionine, selenate, or selenite.

Absorption

Selenium, in organic and inorganic forms, is efficiently absorbed. The duodenum appears to be the primary absorptive site, with some absorption also occurring in the jejunum and ileum.

Selenoamino acid absorption, which occurs through amino acid transport systems, is estimated to be over 80%. Selenomethionine, however, is thought to be better

absorbed than selenocysteine. Selenite absorption has been shown to exceed 85% in some studies [2]. Selenate is thought to be better absorbed than selenite [3]. Figure 12.15 depicts selenium absorption into intestinal cells.

Factors Influencing Selenium Absorption Factors enhancing selenium absorption include vitamins C, A, and E, as well as the presence of reduced glutathione in the intestinal lumen. Heavy metals (such as mercury [4]) and phytates are thought to inhibit selenium absorption through chelation and precipitation.

Transport

Following absorption from the intestine, selenium is bound to transport proteins for travel through the blood to the liver and other tissues. In the blood, selenium binds to sulphhydryl groups in α - and β -globulins such as very low density lipoproteins and low-density lipoproteins, respectively. The selenocysteine-containing plasma protein selenoprotein P contains the majority (>50%) of selenium as selenocysteine in the plasma, but whether the protein releases the selenium for tissue uptake is unclear. Selenoprotein P is described further in the “Functions and Mechanisms of Action” section.

Uptake and Storage

The mechanism by which selenium is freed from plasma transport proteins and taken up by tissues is not known. Tissues containing relatively high selenium concentrations include the thyroid gland, kidney, liver, heart, pancreas, and muscle. The lungs, brain, bone, and red blood cells also contain selenium. Elevated tissue

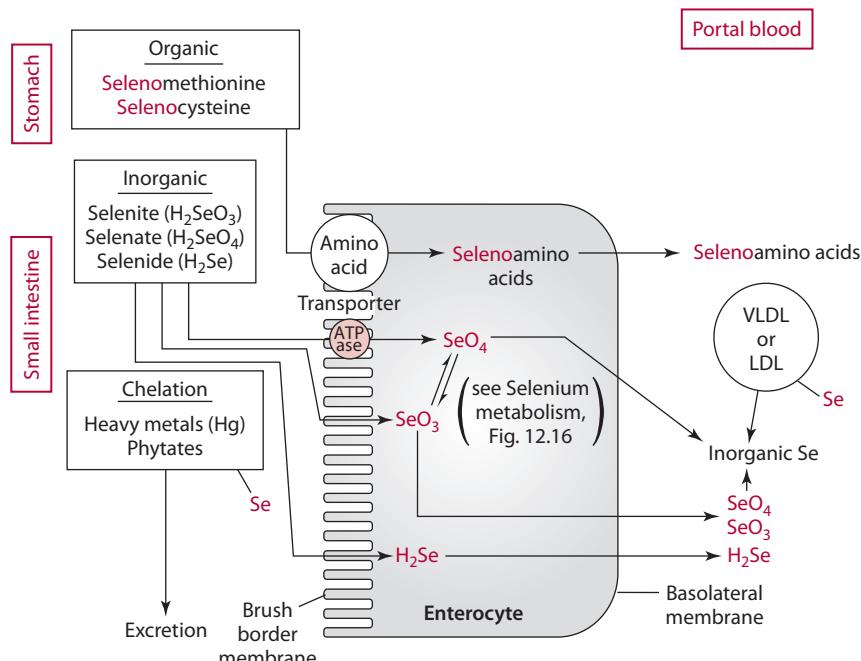


Figure 12.15 Overview of selenium absorption and transport.

concentrations of selenium have resulted when selenium was administered as selenomethionine rather than as selenite. The reverse is true with respect to the uptake of selenium by one of the main selenium-containing metalloenzymes, glutathione peroxidase. That is, ingesting selenium in inorganic forms, such as selenite, causes more of the mineral to be incorporated into glutathione peroxidase than ingesting the organic form, selenomethionine, does [4].

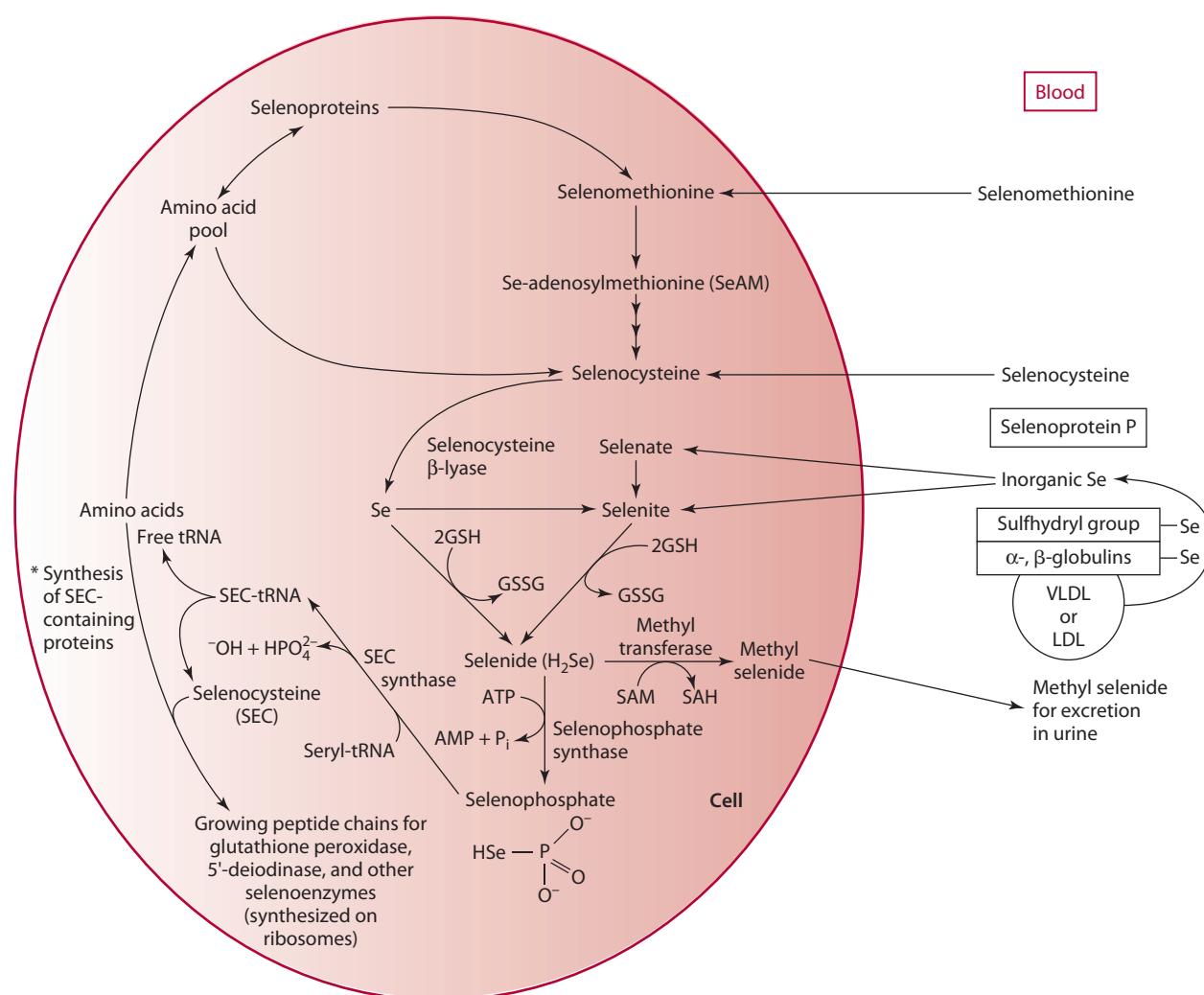
Metabolism

Within tissues such as the liver, selenoamino acids and inorganic forms of selenium undergo metabolism.

Selenomethionine, which is derived from the diet, may be either stored as selenomethionine in an amino acid pool, used for protein synthesis just as the amino acid

methionine is used, or catabolized to ultimately yield selenocysteine. Selenomethionine metabolism is similar to methionine metabolism and is shown in Figure 12.16.

Selenocysteine, which is derived either from selenomethionine metabolism or from the diet, may be degraded by selenocysteine β -lyase to yield free elemental selenium. Free selenium is typically converted (reduced non-enzymatically) in the body to selenide, with hydrogens provided by glutathione or other thiols. Selenide, in turn, can be methylated and excreted in the urine or can be converted by selenophosphate synthase into selenophosphate, an important intermediate in the synthesis of the body's selenium-dependent enzymes. Interestingly, although selenocysteine is required for selenium-dependent enzyme function, it cannot be used directly from diet or from selenomethionine degradation. Instead,



* Synthesis of selenocysteine-containing proteins begins with selenophosphate and the amino acid serine, which is esterified to a specific transfer (t)RNA to form seryl tRNA^{SEC}_{UCA}. Selenocysteine synthase replaces the hydroxy group of serine with a HSe⁻ from selenophosphate to form SEC-tRNA^{SEC}_{UCA} or SECtRNA for short. SEC-tRNA delivers selenocysteine to the growing peptide chains of the various SEC-containing proteins like glutathione peroxidase, iodothyronine 5'-deiodinase, thioredoxin reductase, among others.

Figure 12.16 Selenium metabolism.

selenocysteine must be synthesized in the body from serine while the serine is attached to transfer (t) RNA and selenophosphate (Figure 12.16).

Inorganic selenium also undergoes metabolism (Figure 12.16). Selenate from the diet may be converted in the body to selenite, which is further metabolized to selenodiglutathione and subsequently to selenide. Selenide is metabolized as described above to generate methylated forms for excretion or to form selenophosphate for further use in the synthesis of selenoenzymes, such as those covered in the next section.

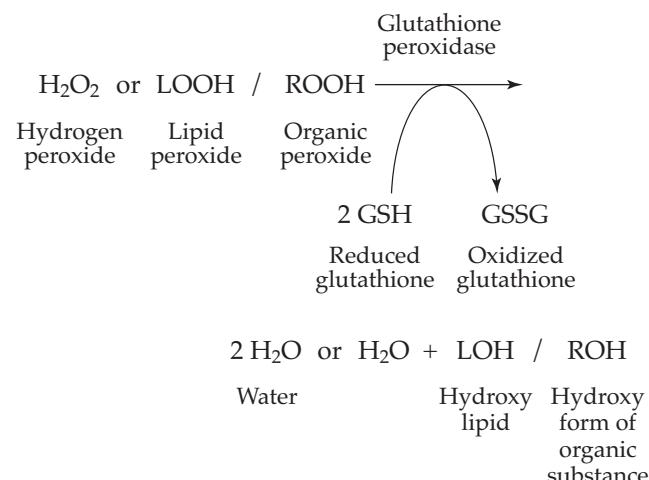
FUNCTIONS AND MECHANISMS OF ACTION

Various incompletely understood roles have been postulated for selenium in mammalian metabolism. Some of the less defined roles are its involvement in maintaining or inducing the cytochrome P₄₅₀ system, in pancreatic function, in DNA repair and enzyme activation, in immune system function, and in detoxifying heavy metals. The better-characterized roles of selenium are related to its functions as an integral part of specific enzymes in the body, although only a few selenium-dependent enzymes are well studied. The next sections describe some of the selenium-dependent enzymes.

Glutathione Peroxidase (GPX)

One of the most clearly established functions of selenium is as an essential cofactor for the enzyme glutathione peroxidase. Several glutathione peroxidase enzymes (designated GPX followed by a number) have been characterized, and each catalyzes the same basic reaction but in different tissues. GPXs 1–4 are selenium dependent, containing four selenocysteine residues. GPX1 and GPX4 are found in most body tissues but most notably in the liver, kidney, and red blood cell. GPX2 is found mainly in the gastrointestinal tract and liver. GPX3 is found mainly in the plasma (extracellular), kidney, and thyroid gland. Within tissues, glutathione peroxidase is found mainly (~70%) in the cytosol of cells and to a lesser extent (~30%) in the mitochondrial matrix; however, GPX4 is found predominantly associated with cell membranes. GPX3 and selenoprotein P together are thought to account for over 90% of the selenium in the plasma.

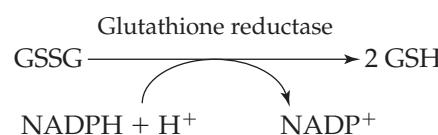
Glutathione peroxidase catalyzes the removal from tissues of hydrogen peroxides (H_2O_2) and hydroperoxides. GPX4 functions mainly to remove organic hydroperoxides (designated LOOH for a lipid hydroperoxide or ROOH for a general organic hydroperoxide) associated with membranes. Glutathione, a tripeptide of glycine, cysteine, and glutamate found in most body cells, is needed in its reduced form for the GPX-catalyzed reaction and furnishes the reducing equivalents, as shown in the following reactions.



Organic peroxides are derived from nucleic acids and other molecules, including unsaturated fatty acids. A peroxide derived from fatty acids is considered a lipid (rather than organic) peroxide. Hydrogen peroxides are generated in many cells throughout the body as part of normal metabolism and may be generated in large amounts by activated white blood cells as they phagocytize foreign substances. The reaction, catalyzed by glutathione peroxidase, neutralizes or eliminates hydrogen peroxide and organic (including lipid) peroxides. In fact, glutathione peroxidase is more active than catalase in reducing organic peroxides and hydrogen peroxides. If not removed, these peroxides typically damage cellular membranes and other cell components.

Selenium availability affects GPX activity, concentrations, and mRNA levels. With selenium deficiency, GPX mRNA concentrations, GPX concentrations, and GPX activity are diminished [1,5,6]. With inadequate selenium, available selenium is shifted to other, more critical selenoproteins, such as selenoprotein P. With selenium supplementation, GPX mRNA increases rapidly to control levels, and enzyme activity gradually increases. The generation of hydrogen peroxides and lipid and organic peroxides—and the coordinated roles of selenium (as part of glutathione peroxidase) and vitamin E, iron (as catalase and myeloperoxidase), and zinc and copper (as superoxide dismutase), which also function as antioxidants to prevent free radical-induced cell damage—are detailed in the Perspective at the end of Chapter 10.

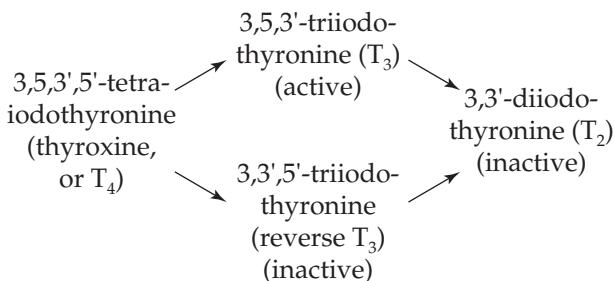
The GSSG that is formed as a result of GPX activity must be regenerated back to its reduced form (GSH). This regeneration is imperative for cells to maintain appropriate redox states. Glutathione reductase, a flavoenzyme, catalyzes this reduction in a reaction dependent on $NADPH + H^+$, which is derived from the hexose monophosphate shunt. The regeneration of reduced glutathione is shown here:



Iodothyronine 5'-Deiodinases (ID1 or DI)

Selenium is also necessary for iodine metabolism and has been suggested to regulate thyroid hormone production [7]. Iodothyronine 5'-deiodinases are selenocysteine-containing proteins with a selenocysteine present at the active site. Three types of 5'-deiodinases have been characterized. Type 1 is found mainly in the thyroid gland and liver, and types 2 and 3 are found in tissues such as skin, pituitary, adipose, and brain.

5'-deiodinases catalyze the deiodination (removal of iodine) from the 5 or 5' positions of thyroid hormones and some of their metabolites. For example, deiodinases types 1 and 2 convert the thyroid hormone thyroxine (T_4), which is secreted from the thyroid gland, to 3,5,3'-triiodothyronine (T_3). Type 1 deiodinase, which catalyzes this reaction in the liver (among other tissues such as the kidney, and the pituitary and thyroid glands), provides T_3 for release into the blood and circulation in the body. Type 2 deiodinase provides for the production and use of T_3 within specific tissues. T_3 is the body's primary hormonal regulator of metabolism as well as of normal growth and development.

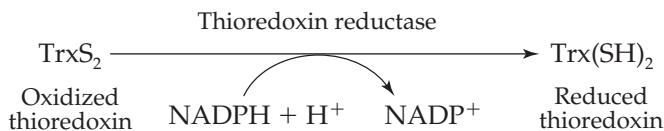


Once made, T_3 can be deiodinated, by type 3 deiodinase to T_2 (also called 3,3'-diiiodothyronine). Other reactions also can occur. If T_3 is not needed, then, for example, T_4 may be converted by type 3 deiodinase into reverse T_3 , an inactive metabolite. For further information regarding thyroid hormone metabolism, see the section of this chapter on iodine.

Thioredoxin Reductase (TrxR or TRR)

Thioredoxin reductase is a flavoenzyme (FAD) that, like GPX and deiodinase, contains selenocysteine at its active site. The enzyme, found in the blood as well as within cells and tissues, helps maintain the body's and the cell's redox state by acting on thioredoxin as well as other substrates. Specifically, thioredoxin reductase transfers reducing equivalents from NADPH through its bound FAD to reduce disulfide bonds (S-S) within the oxidized form of its substrate thioredoxin (Trx). Thioredoxins as well as glutaredoxins are small peptides (also called dithiols because of the presence of two sulfhydryl groups) found in cells. Thioredoxins, $[Trx(SH)_2]$ in its reduced state, function with thioredoxin reductase and NADPH

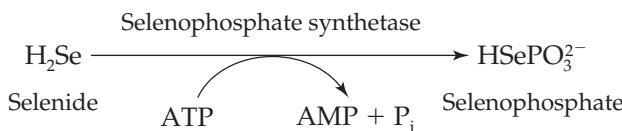
as a protein disulfide reducing system. In other words, the thioredoxins provide oxidized compounds with hydrogens (i.e., reducing equivalents). The reaction catalyzed by thioredoxin reductase, shown below, is similar to that catalyzed by glutathione reductase.



The thioredoxin system is involved in oxidation-reduction roles and also helps to modulate intracellular signalling cascades, inhibit apoptosis, and regulate cell growth [8]. For example, thioredoxin reduces transcription factors such as activator protein (AP)1 and nuclear factor κ B to affect their DNA-binding capacities.

Selenophosphate Synthetase (SPS)

At least two forms of selenophosphate synthetase have been identified in the body. One form (called SPS1) does not contain selenocysteine and is thought to recycle selenium from selenocysteine. The SPS2 isoform, which contains selenocysteine, catalyzes the synthesis of selenophosphate from selenide, as shown here:



Selenophosphate is a key compound needed in the body to synthesize other selenocysteine-containing proteins or enzymes (Figure 12.16) such as glutathione peroxidase, deiodinase, thioredoxin reductase, selenoprotein P, and others.

Selenoprotein P (SEL P)

Selenoprotein P, a glycoprotein, is synthesized mostly in the liver and to lesser degrees in the kidneys, heart, and lungs. It is the major selenium-containing protein in the blood; however, it also is found associated with capillary endothelial cells. Selenoprotein P, unlike most selenoenzymes (which contain ~1 to 4 selenium atoms as selenocysteine), contains up to ten selenocysteine residues. However, under conditions in which selenium is limited, selenoprotein P may be synthesized with fewer selenocysteine residues. In other words, instead of having ten selenocysteines, selenoprotein P may only have two or three or so selenocysteines if sufficient selenium is not available in the cells. Moreover, when selenium is limited, selenoprotein P appears to preferentially receive selenium over other selenoenzymes such as glutathione peroxidases [9].

Selenoprotein P is thought to function in the body as an antioxidant, especially in removing the damaging peroxynitrite (ONOO^-) radical. Peroxynitrite is synthesized

by activated white blood cells from superoxide radicals (O_2^-) and nitrogen monooxide (NO^\bullet) [9–11]. If not inactivated, peroxynitrite, for example, can cause DNA single-strand breaks and lipid peroxidation.

Selenoprotein W (SEL W)

Selenoprotein W, also a selenocysteine-containing protein, is found mostly in the cytosol of cardiac and skeletal muscle. In these tissues, selenoprotein W may be found bound to reduced glutathione through a cysteine residue. The function of this protein is unclear at present, but it is speculated to have antioxidant roles in the body [12–14].

Methionine R Sulfoxide Reductase (SEL R)

Methionine R sulfoxide reductase also contains selenocysteine. The enzyme reduces methionine R sulfoxides, which are generated in proteins when free radicals cause oxidation of methionine residues. The presence of the sulfoxide within the damaged protein renders the protein unable to perform its normal function.

Other Selenoproteins

Several other selenoproteins containing at least one selenocysteine have been identified, but little is known about their functions. Selenoprotein 15 (SEP 15), for example, is thought to function as a chaperone in cells, and may also be involved with the folding of proteins, which may be necessary before a protein is secreted from a cell and into the blood. Selenoprotein S (SEL S) is thought to be involved with the processing and removing of misfolded proteins in cells. Selenoproteins H, K, M, and N also have been identified, but their functions are not known.

INTERACTIONS WITH OTHER NUTRIENTS

Iron and copper deficiencies affect selenium function in the body. Iron deficiency decreases the synthesis of hepatic glutathione peroxidase and reduces tissue selenium concentrations [15]. Copper deficiency has been shown to decrease the activities of both glutathione peroxidase and 5'-deiodinase [16,17]. The mechanism or mechanisms by which iron and copper act are unclear.

Dietary methionine intake can also affect selenium. A problem occurs if the body's selenium is available only as selenomethionine. With selenium present in the body only as selenomethionine, the selenium then becomes available only as proteins are degraded in the course of normal turnover [18].

EXCRETION

Selenium is excreted from the body almost equally in the urine and feces. Excretion is thought to be the means by which selenium homeostasis is maintained. Major urinary metabolites of selenium include a selenosugar methyl

seleno-N-acetyl D-galactosamine [$CH_3Se\text{-GalN}$], methylselenol [CH_3SeH], dimethylselenide [$(CH_3)_2Se$], and trimethylselenium [$(CH_3)_3Se^+$] [19].

Selenium losses through the lungs and skin also contribute to daily selenium excretion. Pulmonary elimination of selenium, usually associated with ingestion of large amounts of the mineral, occurs by exhalation of dimethylselenide, which is quite volatile and has a garlicky odor.

RECOMMENDED DIETARY ALLOWANCE

Recommendations in the form of an estimated safe and adequate daily dietary intake were developed for selenium in 1980. These recommendations were based primarily on calculations of selenium requirements for animals and were set at 50 to 200 $\mu\text{g}/\text{day}$ [20]. In 1989, RDAs for selenium were established (70 μg for men and 55 μg for women) [21]. The RDAs have subsequently been modified as additional studies have been conducted over the last decade. In 2000, the Food and Nutrition Board set an RDA for selenium for adult men and women of 55 $\mu\text{g}/\text{day}$ [22]. Based mostly on balance studies as well as on repletion studies of men with selenium deficiency in regions of China, the requirement for selenium for adults was determined to be 45 μg . The requirement was based on calculation of the amount of selenium necessary for plateau concentrations of selected selenoproteins in the plasma. To set the RDA, a 20% coefficient of variation was added, and the final number was rounded to the nearest five. RDAs for selenium for pregnancy and lactation were set at 60 μg and 70 μg , respectively [22]. The additional selenium is needed for fetal deposition during pregnancy and for inclusion in milk during lactation [22]. The inside cover of the book provides RDAs for selenium for other age groups. Recent studies suggest that the RDA for selenium for adults may be suboptimal [23].

DEFICIENCY

Selenium deficiency has been linked to a number of livestock animal diseases and also to the regional human diseases such as Keshan disease and Kashin-Beck's disease in China [24]. Keshan disease is characterized by cardiomyopathy involving cardiogenic shock, congestive heart failure, or both, along with multifocal necrosis of heart tissue, which becomes replaced with fibrous tissue [24]. Coxsackie virus appears to be a cofactor in the development of Keshan disease. In the absence of sufficient selenium, mutations occur in benign strains of the virus. These mutations cause the virus to become virulent; the presence of the virus is thought to account for some of the symptoms of Keshan disease [25]. Kashin-Beck's disease is characterized by

osteopathia involving degeneration and necrosis of the joints and of epiphyseal-plate cartilages of the legs and arms [24]. Several factors, including selenium deficiency, are thought to contribute to the development of Kashin-Beck's disease.

Selenium deficiency also has been observed in people receiving total parenteral nutrition [26–28]. Major symptoms of deficiency included poor growth, muscle pain and weakness, loss of pigmentation of hair and skin, and whitening of nail beds. Poor growth may be associated with the role of selenium in thyroid hormone metabolism. Serum selenium concentrations have been inversely associated with heart disease risk and incidence of some cancers [29–31]. Some studies have suggested that a daily selenium intake of 200 µg may diminish the risk of these diseases [30].

TOXICITY

Selenium toxicity, also called selenosis, has been observed both in miners and in people who consume excess selenium from supplements. Physical manifestations as well as biochemical abnormalities may occur, depending on amounts ingested. Signs and symptoms of toxicity include nausea, vomiting, fatigue, diarrhea, hair and nail brittleness and loss, paresthesia, interference in sulfur metabolism (primarily oxidation of sulfhydryl groups), and inhibition of protein synthesis [32]. Acute poisoning from gram amounts of selenium is lethal, with damage occurring to most organ systems [32]. A tolerable upper intake level of 400 µg/day has been set by the Food and Nutrition Board [22]. The lowest observed adverse effect level (LOAEL) for selenium is 910 µg [21].

ASSESSMENT OF NUTRITION

The concentration of selenium in the blood is thought to be a reflection or function of dietary intake within a specific range. For plasma selenium concentrations, a value of 7 µg/dL (0.8 micromol/L) appears to be the cut-off [22,32]. If a person's plasma selenium concentration is <7 µg /dL, dietary selenium affects the plasma selenium concentration. When plasma concentrations exceed this value, factors other than diet also affect plasma concentrations [22].

The activities and concentrations of selenoproteins also have been used to assess selenium status. Selenoprotein P and glutathione peroxidase in tissues (GPX1) and in the plasma (GPX3) are commonly used. Selenoprotein P concentrations are thought to be a better indicator of selenium status than glutathione peroxidase [34]. Selenoprotein P and glutathione peroxidase activity (like serum or plasma selenium concentrations) plateau as selenium intake increases, thus serving as an index of selenium status in populations with low intake [21,22,33].

Toenail clippings also appear to reflect selenium status for up to 1 year before sampling; however, nails and hair are influenced by the forms of selenium ingested and, in the case of hair, by its color and by contamination from shampoos and other products [20,35–37]. Urinary selenium concentration may reflect status, but it can be affected by diet. It has also been shown to identify selenium toxicity but may be proportional to status [20,32,36,37].

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Chromium

Chromium, a metal, exists in several oxidation states from Cr²⁺ to Cr⁶⁺. The metal has ubiquitous presence—found in air, water, and soil. Trivalent chromium, or Cr³⁺, is the stablest of the oxidation states and often binds to ligands containing nitrogen, oxygen, or sulfur to form hexacoordinate or octahedral complexes. The trivalent form of chromium is thought to be the most important form in humans. The chromium content of the human body is estimated at ~4 to 6 mg.

SOURCES

In foods, chromium exists in the trivalent form (Cr³⁺). Good sources of dietary chromium include meats, fish, and poultry (especially organ meats) and grains (especially

whole grains) [1,2]. Other foods provide variable amounts of chromium. Examples of foods containing relatively large amounts of chromium include cheese, dark chocolate, selected vegetables including mushrooms, green peppers, green beans, and spinach; selected fruits such as apples, bananas, orange and grape juices; and various condiments and spices (cinnamon, cloves, bay leaves, turmeric); as well as tea, beer, and wine [1,2]. Brewer's yeast is notable because of its suspected high content of the biologically active organically complexed form of chromium often called glucose tolerance factor (GTF).

Food processing and refining can affect the chromium content of foods. Refining of sugar, for example, diminishes chromium. Thus, molasses and brown sugar are higher in chromium than white sugar. In contrast, chromium is easily solubilized from stainless steel cookware or cans into acidic foods. Thus, use of stainless steel cookware may increase the amount of chromium in food [3].

ABSORPTION, TRANSPORT, AND STORAGE

Absorption

In acidic solutions, as would be found in the stomach, Cr³⁺ is soluble and may form complexes with ligands. Chromium is thought to be absorbed throughout the small intestine, especially in the jejunum [4]. Although the mode of absorption is still not known, chromium is thought to be absorbed either by diffusion or by a carrier-mediated transporter. About 0.4% to 2.5% of chromium intake is absorbed into intestinal cells for use by the body [5–8].

Factors Influencing Chromium Absorption

Like that of other trace minerals, chromium absorption may be influenced by dietary factors.

Enhancers of Chromium Absorption Within the stomach, amino acids or other ligands may chelate inorganic chromium. Amino acids such as phenylalanine, methionine, and histidine, for example, act as ligands to improve chromium absorption [9]. Picolinate also acts as a ligand for chromium. Such chelations typically help chromium remain soluble and prevent olation (see next paragraph) once it reaches the alkaline pH of the small intestine. Lipophilic compounds such as picolinate are also beneficial, enhancing absorption through a cell's lipid membranes. Vitamin C also appears to enhance chromium absorption. Consuming 1 mg chromium (as chromium chloride) along with 100 mg ascorbate was associated with greater plasma chromium concentrations than those attained by ingesting chromium without ascorbate [10–12].

Inhibitors of Chromium Absorption Inorganic chromium in a neutral or alkaline environment reacts with hydroxylions (OH⁻), which readily polymerize to form high-molecular weight compounds in a process called olation. This reaction results in precipitation of chromium and thus

reduced absorption. Antacids significantly reduce blood and tissue chromium concentrations by decreasing absorption [10]. Phytates, found mostly in grains and legumes, also diminish chromium absorption.

Transport

In the blood, inorganic Cr^{3+} binds competitively with transferrin and is transported along with iron bound to transferrin. If transferrin sites are unavailable for chromium, albumin is thought to transport chromium.

Globulins and possibly lipoproteins also are thought to transport the mineral if present in very high concentrations. Some chromium also may circulate unbound in the blood. How organically complexed chromium is transported in the blood remains uncertain.

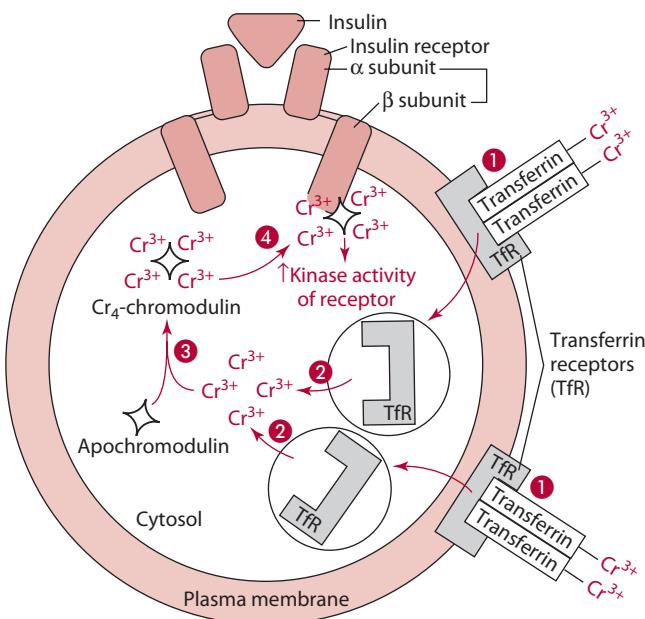
Storage

The body contains ~4 to 6 mg chromium [13]. Tissues especially high in chromium include the kidneys, liver, muscle, spleen, heart, pancreas, and bone. Tissue chromium concentrations have been shown to decline with age [14]. Chromium is thought to be stored in tissues with ferric iron because of its transport by transferrin.

FUNCTIONS AND MECHANISMS OF ACTION

Chromium is known to potentiate the action of insulin; however, the mechanism by which potentiation occurs is still under investigation. For decades, the biological action of chromium has been believed to be attributable to its complexing with nicotinic acid and amino acids to form the organic compound glucose tolerance factor (GTF) [15,16]. GTF was first identified in brewer's yeast, but this factor has never been purified, nor has its exact structure been characterized. Nevertheless, the belief remains that the biologically active molecule is a dinicotinato chromium complex coordinated with amino acids that stabilize the complex. Mertz [15,16] proposed a dinicotinato chromium complex coordinated by amino acid (glutamate, cysteine, and glycine) ligands, which stabilized the complex. GTF, released in response to insulin, was thought to potentiate the actions of insulin possibly by facilitating insulin binding. However, no evidence indicates that chromium is a component of the receptor's subunits or is part of an accessory protein for insulin binding [13,17,18].

More recent studies have shown that chromium may be involved in pancreatic insulin secretion or in insulin receptor production, expression, or activity to potentiate or enhance insulin's effectiveness [17–19]. Insulin is more effective in the presence of chromium than in its absence [19]. The role of chromium in stimulating insulin activity is thought to occur as described next and shown in Figure 12.17. With increased plasma insulin, chromium bound to transferrin is taken into the cells through transferrin



- ① Transferrin delivers Cr^{3+} to transferrin receptors (TfR) on cell membranes.
- ② Cr^{3+} is released inside the cell.
- ③ Four Cr^{3+} atoms complex with chromodulin to form holo chromodulin or $\text{Cr}_4\text{-chromodulin}$.
- ④ $\text{Cr}_4\text{-chromodulin}$ functions to increase the kinase activity of the beta subunit of the insulin receptor, and other cytosolic tyrosine kinases.

Figure 12.17 Proposed role of chromium (Cr^{3+}) as part of chromodulin in potentiating insulin's reactions.

receptors. Within the cells, the released chromium atoms (four) bind to apochromodulin. Apochromodulin is an oligopeptide composed of glycine, cysteine, aspartate, and glutamate. Once the four chromium atoms bind to the apochromodulin, the complex is called holochromodulin ($\text{Cr}_4\text{-chromodulin}$) or chromodulin. Chromodulin also has been referred to as a low-molecular-weight chromium (LMWCr) binding substance.

Chromodulin, but not apochromodulin, binds to the cytosolic beta subunit of the insulin receptor and stimulates (or amplifies) the kinase activity of the insulin receptor. Chromodulin also appears to stimulate the tyrosine kinase activity of other enzymes, which in turn phosphorylate a variety of cytosolic proteins involved in insulin signalling [20–26].

Roles for chromium in glucose and lipid metabolism have been suggested but likely occur because of insulin's effects on multiple enzymes regulating glucose and lipid metabolism. For example, chromium may improve glucose intolerance in people with impaired glucose tolerance if the individual has suboptimal chromium status. Several studies have reported improvements in blood glucose and lipid profiles of people following chromium supplementation, but effects are positive in those with initial suboptimal chromium status [6,15,17,18,27,28]. Chromium as a supplement has been purported to effect changes in body

composition and strength performance. However, most well-controlled studies providing chromium supplementation have shown no significant effects on strength gains, muscle accretion, or fat loss [29–33].

Another proposed role for chromium is in nucleic acid metabolism. Cr³⁺ is thought to be involved in maintaining the structural integrity of nuclear strands and in regulating gene expression [34]. RNA synthesis in vitro, as directed by DNA, is enhanced by chromium binding to the template [35].

INTERACTIONS WITH OTHER NUTRIENTS

Because chromium is transported in the blood bound to transferrin, the primary iron-binding protein, one might surmise that chromium, if given in large amounts, might displace iron from the transferrin. Indeed, ingesting chromium (~200 µg) as chromium chloride and chromium picolinate has been associated with a significant decrease in serum ferritin, total iron-binding capacity, and transferrin saturation in men [31]. Other studies, however, report that chromium picolinate ingestion (924 µg) had no effects on hematologic indexes in men [33].

EXCRETION

Most chromium is excreted from the body in the urine. In fact, urinary chromium represents about 95% of chromium excretion and mainly reflects recent intake (not status). In absolute terms, urinary chromium is ~0.2 to 0.4 µg /day, and 0.5% to 2% of intake is excreted with intakes of 40 µg and 10 µg, respectively [6,8,36–38]. Consumption of diets high in simple sugars (35% simple sugars, 15% complex carbohydrates) has been shown to raise urinary chromium in some subjects by 300% in contrast to consumption of diets high in complex (starch) carbohydrates (35% complex carbohydrates, 15% simple sugars) [39]. In addition to urinary losses, small amounts of chromium are lost with desquamation of skin cells. Fecal chromium represents mostly unabsorbed dietary chromium, not endogenous chromium excreted with the bile into the feces.

ADEQUATE INTAKE

An estimated safe and adequate daily dietary intake (ESADDI) for chromium was first reported in the 1980 RDAs. In 1989, an ESADDI for chromium of 50 to 200 µg/day was recommended for adults [40]. The latest recommendation for chromium is in the form of an adequate intake (AI) and was set based on estimated mean chromium intake in the United States. The AIs for chromium for adult men and women through age 50 years are 35 µg and 25 µg, respectively; these values drop to

30 µg and 20 µg for men and women, respectively, over 50 years of age [7]. During pregnancy and lactation, intakes of 30 µg and 45 µg chromium, respectively, are recommended [7]. AIs for chromium for other age groups are provided on the inside cover of the book.

DEFICIENCY

Chromium deficiency has been described in a couple of people who received intravenous nutrition feeding (total parenteral nutrition) without chromium and without oral food intake. Signs and symptoms of deficiency included weight loss, peripheral neuropathy, elevated plasma glucose concentrations or impaired glucose use (also called insulin resistance, which may be characterized by hyperinsulinemia), and high plasma free fatty acid concentrations. Evidence of impaired glucose tolerance increases among the aged and may be related to inadequate intake of chromium or declines in tissue concentrations [41]. Improved chromium status, if initially suboptimal, results in improved glucose metabolism in people with diabetes and glucose intolerance.

Severe trauma and stress may increase the need for chromium. Stress, for example, elevates the secretion of hormones such as glucagon and cortisol, which alters glucose and ultimately chromium metabolism.

Chromium needs also may be increased in certain diseases, such as diabetes mellitus and heart disease, although a link between chromium and these diseases is not conclusive. Chromium deficiency results in insulin resistance characterized by hyperinsulinemia, a risk factor for heart disease. Mild chromium deficiency also is a risk factor for metabolic syndrome. Metabolic syndrome is a constellation of abnormalities that increases the risk of heart disease, and includes hyperinsulinemia, resistance to insulin-stimulated glucose uptake, glucose intolerance, hypertriglyceridemia (high concentrations of triglycerides in the blood), decreased blood HDL concentrations, and hypertension.

SUPPLEMENTS

Chromium is available in supplement form as inorganic salts, such as with chloride, or as an organic complex, such as with acetate, nicotinic acid alone or with amino acids, or picolinic acid. Although all forms appear to be absorbed and used, the form of the supplement appears to affect tissue concentrations in rats. Chromium picolinate, because of its increased solubility (it is lipophilic), has been touted as superior to other forms of chromium, but it may cause chromosomal damage [42]; other studies have indicated potential for organ damage [43–48]. Advertisements suggesting that the use of chromium picolinate may help a person lose fat and gain muscle (lean body) mass do not appear to be entirely supported by scientific research [29–33].

TOXICITY

Oral supplementation of up to about 1,000 µg of chromium as Cr³⁺ appears to be safe [7,46]. However, chromium (Cr³⁺) picolinate has been shown to produce chromosomal damage in hamster cells [42]. In addition, chromium picolinate providing between 600 and 2,400 µg chromium has been associated with renal failure and hepatic dysfunction [43,44].

Toxicity is associated with exposure to the hexavalent form (Cr⁶⁺) of chromium that may be absorbed through the skin, enter the body through inhalation, or be ingested. Inhalation of or direct contact with hexavalent chromium may result in respiratory disease or in dermatitis and skin ulcerations, respectively. Liver damage may also occur. Cr⁶⁺ ingested orally is about 10 to 100 times more toxic than Cr³⁺ [47]. Ingesting chromic acid (CrO₃), which contains hexavalent chromium, has resulted in severe acidosis, gastrointestinal hemorrhage, hepatic injury, renal failure, and death [45].

The no observed adverse effect level (NOAEL) for chromium Cr³⁺ is set at 1,000 µg/day [48]. No tolerable upper intake level has been established by the Food and Nutrition Board to date.

ASSESSMENT OF NUTRITUDE

No specific tests are currently available to determine chromium status. Although a plasma chromium level of ~0.5 ng/mL is considered normal, the chromium content of physiological fluids is not indicative of status [36]. Fasting plasma chromium is not in equilibrium with tissue chromium. Responses of plasma chromium to an oral glucose load are inconsistent. Urinary chromium appears to reflect only recent intake, not status [36]. Hair chromium concentrations may indicate the status of a large population but not of individuals [15]. Relative chromium status has been evaluated retrospectively through following the effects of chromium supplementation on various parameters such as blood glucose and lipids, but this assessment is not valid in determining absolute nutriture.

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Iodine

Iodine, a nonmetal, typically is found and functions in its ionic form, iodide (I^-). Hence, the term *iodide* is used throughout this section about this trace element. About 15 to 20 mg iodide is found in the human body.

SOURCES

The iodide concentration in foods is extremely variable because, as is so often the case, it reflects the regionally variable soil concentrations of the element and the amount and nature of fertilizer used in plant cultivation. Thus, the iodide content of grains, vegetables, and fruits varies with the iodide content of the soil, and the iodide content of meats depends on the iodide of the soil and plants that the animals ate. The amount of iodide in drinking water is an indication of the iodide content of the rocks and soils of a region and closely parallels the incidence of iodine deficiency among the inhabitants of that region. For example, the iodide content of water from goitrous areas in India,

Nepal, and Ceylon ranged from 0.1 to 1.2 mg/L, compared to 9.0 mg/L found in nongoitrous Delhi [1]. In the United States, before salt was fortified with iodine in the 1920s, people living in the Great Lakes and Rocky Mountain areas had iodine-poor diets.

Iodide is found in seafoods; however, large differences in iodide content exist between seawater fish and freshwater fish. Edible sea fish contain about 30 to 300 $\mu\text{g}/100\text{ g}$, in contrast to only 2 to 4 $\mu\text{g}/100\text{ g}$ freshwater fish. Other protein-rich foods also supply iodide. Milk and yogurt provide about 60–80 $\mu\text{g}/\text{cup}$. An egg, for example, provides about 28 μg iodide, and meats generally provide about 25–35 $\mu\text{g}/100\text{ g}$. Beans, such as navy beans, also contain iodide, about 35 $\mu\text{g}/\text{one-half cup}$. An additional source of iodide is breads and grain products made from bread dough. Dough oxidizers or conditioners contain iodates (IO_3^-) as food additives to improve cross-linking of the gluten [2]. Breads and cereal products generally provide about 10 $\mu\text{g}/100\text{ g}$. Iodized salt (1/4 teaspoon) supplies about 68 μg iodide. Restricting salt intake (as may be necessary for people being treated for hypertension) may negatively affect iodine status [3].

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Dietary iodine (I) is either bound to amino acids or found free, primarily in the form of iodate (IO_3^-) or iodide (I^-) (Figure 12.18). During digestion, organic bound iodine may be freed. Iodate, for example from breads, is usually reduced to iodide by glutathione [4]. Small quantities of iodinated amino acids and other organic forms of iodide that escape digestion may be absorbed, but not as efficiently as the iodide ion. The thyroid hormones

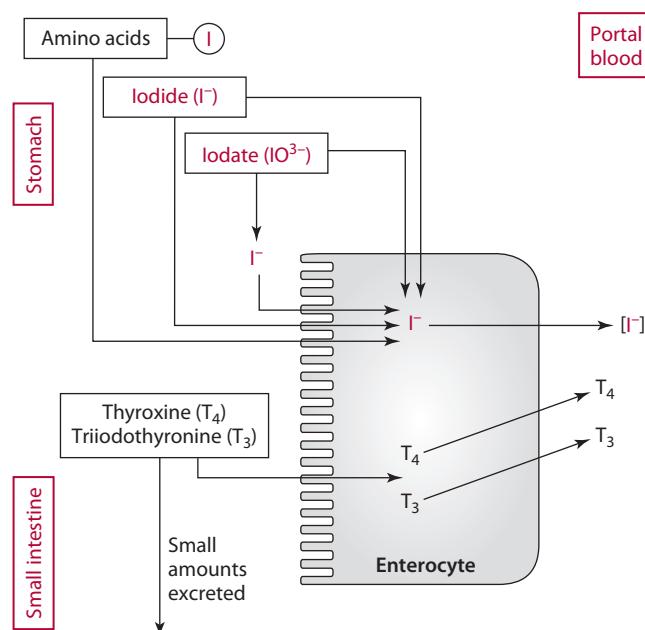


Figure 12.18 Digestion and absorption of iodine.

thyroxine (T_4) and triiodothyronine (T_3) also are absorbed unchanged, with a bioavailability of about 75%, which allows T_4 medication to be administered orally [5].

Iodide is absorbed rapidly and completely throughout the gastrointestinal tract, including the stomach. Thus, very little iodide appears in the feces.

Following absorption, free iodide appears in the blood (Figure 12.18). Iodide is distributed throughout the extracellular fluid, from which it is capable of permeating all tissues. The element selectively concentrates, however, in the thyroid gland with lesser amounts found in ovaries, placenta, skin, and salivary, gastric, and mammary glands.

The thyroid gland traps iodide most aggressively, doing so by way of a sodium-dependent, active transport system against an iodide gradient that is often 40 to 50 times the plasma concentration. The thyroid gland contains 70% to 80% of the total body iodide and takes up about 120 μg of iodide per day.

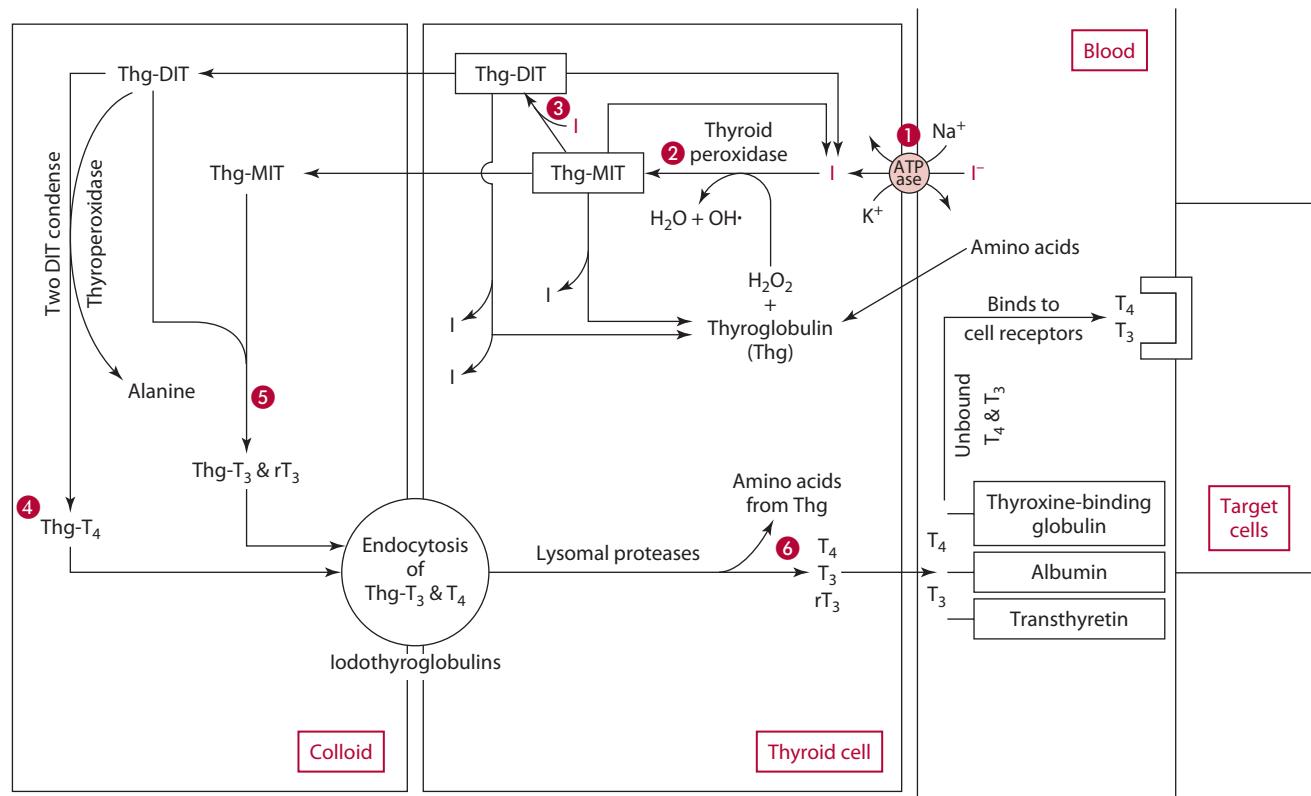
Because the thyroid gland and its synthesis of the thyroid hormones are the focal points of iodide metabolism, information on the transport of iodide into nonthyroidal tissue

is sparse. However, uptake by other tissues such as salivary glands likely proceeds by an active transport mechanism.

FUNCTIONS AND MECHANISMS OF ACTION

The main function of iodide is for the synthesis of the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) by the thyroid gland. The thyroid gland is made of multiple acini, also called follicles. The follicles are spherical in shape and are surrounded by a single layer of thyroid cells. The follicles are filled with colloid, a proteinaceous material. Both amino acids and iodide are needed to synthesize thyroid hormones. The events in thyroid hormone synthesis are shown in Figure 12.19 and described here:

- The thyroid cells actively collect iodide from the blood. In fact, the thyroid gland must trap about 60 mg of iodide daily against a steep gradient of the element to ensure an adequate supply of hormones [6]. The trapping mechanism operates through an Na^+/K^+ -ATPase pump (Figure 12.19) [7].



① I^- is actively transported into the thyroid cell.

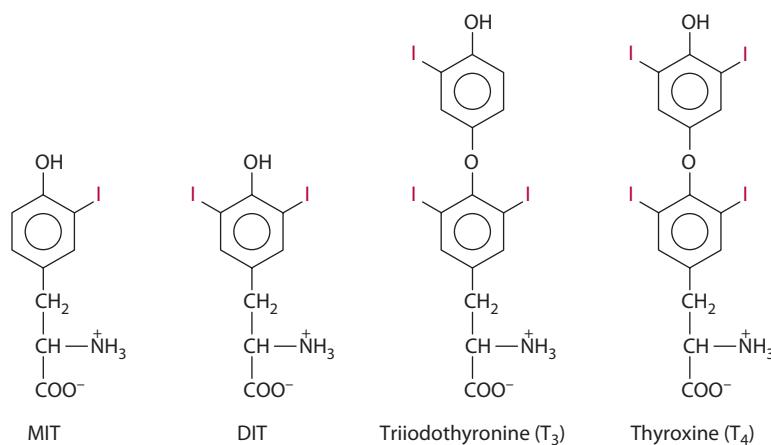
② I is bound to a tyrosine residue on thyroglobulin to form thyroglobulin 3, 5-diiodotyrosine (Thg-MIT).

③ Thg-MIT is iodinated to form Thg-DIT, thyroglobulin 3, 5-diiodotyrosine, which ④ condenses with another Thg-DIT in the colloid to form Thg-T₄.

⑤ Thg-DIT also can condense with Thg-MIT to form Thg-T₃ and reverse (r)T₃.

⑥ T₄ and T₃, active thyroid hormones, are released into the blood following endocytosis of Thg-T₃ and Thg-T₄ back into the thyroid cell and hydrolysis of the Thg by proteases.

Figure 12.19 Overview of iodine intrathyroidal metabolism and hormonogenesis, and thyroid transport and cellular uptake.

**Figure 12.20** The structures of MIT, DIT, T₃, and T₄.

- Once within the cell, iodide (I⁻) is oxidized to iodine (I), which is then bound to the number 3 position of tyrosyl residues of the glycoprotein thyroglobulin (a process called organification of the iodine). The binding of iodine to the tyrosyl residues of thyroglobulin (Thg) is catalyzed by thyroperoxidase and generates thyroglobulin-3-monooiodotyrosine (Thg-MIT) (Figure 12.19). Hydrogen peroxide acts as the electron acceptor.
- Next, MIT is iodinated in the number 5 position to form thyroglobulin-3,5-diiodotyrosine (Thg-DIT). In the colloid, two DITs condense or couple to form Thg-3,5,3',5'-tetraiodothyronine (Thg-T₄) with the elimination of an alanine side chain. Thyroperoxidase catalyzes this coupling reaction.
- DIT also condenses or couples with MIT to form 3,5,3'-triiodothyronine (T₃) and reverse T₃(rT₃).
- DIT and MIT not used for thyroid hormone synthesis in the thyroid cells are deiodinated, and the iodine is made available for recycling in the formation of new iodothyroglobulin. The structures of MIT, DIT, thyroxine (T₄) and 3,5,3'-triiodothyronine (T₃) are shown in Figure 12.20.

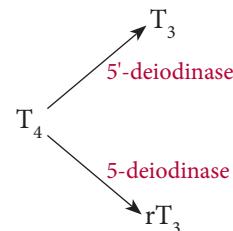
Transport of Thyroid Hormones in the Blood

To release the thyroid hormones into the blood, iodothyroglobulin must be resorbed in the form of colloid droplets by endocytosis back into the thyroid cell (Figure 12.19). Within the thyroid cell, the iodothyroglobulin (Thg-T₄ and Thg-T₃) is hydrolyzed by lysosomal proteases, and T₄ and T₃ are released into the blood. In the blood, T₄ and T₃ associate with transport proteins and are distributed to target cells in peripheral tissues.

Three transport proteins bind and transport T₄ and T₃ in the blood. Thyroxine-binding globulin, found in the plasma, has the smallest capacity but the greatest affinity for T₄ and T₃. Albumin and transthyretin (also called prealbumin) also transport the thyroid hormones. A very small fraction (<0.1%) of the blood T₄ and T₃ is not bound to transport proteins, and it is this free form that is available to the cell receptors and that therefore is hormonally

active. The plasma concentration of T₄ is nearly 50 times that of T₃, but T₃ is many times more potent on an equal molar basis. For a more in-depth description of thyroid hormone synthesis, see the reviews by Visser [8] and Vanderpas [9].

Several tissues—the liver, kidney, brain, pituitary, and brown adipose tissue, to name a few—can deiodinate T₄ to generate T₃ and rT₃. Most T₃ in the blood has been synthesized in the liver from T₄. A selenium-dependent 5'-deiodinase generates T₃, and a 5-deiodinase generates rT₃. Conversion of T₄ to T₃ is impaired with selenium deficiency [10].



The multiple effects of the thyroid hormones result from the hormones' occupancy of nuclear receptors, with subsequent effects on gene expression. The receptors appear to be the same in all tissues, binding T₃ more avidly than T₄ and requiring fivefold to sevenfold higher concentrations of T₄ to achieve comparable physiological effects. Zinc may play a role in the binding of the zinc fingers of the receptor protein (which in turn is influenced by thyroid hormones) to the DNA.

Although mechanisms of action of the thyroid hormones are unclear, biological effects are in response to increased mRNA and protein synthesis triggered by the hormone receptor attachment. Numerous hypotheses for mechanisms have been proposed, including modulation of (Na⁺/K⁺-ATPase) transport systems, adrenergic receptor sensitivity, and neurotransmitters. The review by Sterling [11] provides more comprehensive reading on this topic.

The effects of thyroid hormones on metabolism are many and varied. Thyroid hormones stimulate the basal rate of metabolism, oxygen (O₂) consumption, and heat production and are necessary for normal nervous system

development and linear growth. Directly or indirectly, most organ systems are under the influence of these hormones.

INTERACTIONS WITH OTHER NUTRIENTS

One well-established interaction is that between iodide and goitrogens. Substances that interfere with iodide metabolism in any way that inhibits thyroid hormonogenesis are called goitrogens because their effect is to secondarily augment TSH release and consequently thyroid gland enlargement. Goitrogens may affect iodide uptake by the gland, organification of the iodide, or hormone release from the thyroid cells.

Most goitrogenic compounds act by competing with iodide in its active transport into the thyroid cells. Halide ions such as bromide (Br^-) and astatide (At^-) function in this way, as do thiocyanate (SCN^-), perrhenate (ReO_4^-), and pertechnetate (TcO_4^-). Perchlorate (ClO_4^-), along with perrhenate and pertechnetate, interferes with organification as well as uptake. Lithium (Li^+), used to treat some psychiatric disorders, inhibits hormone release from the gland. Other classes of goitrogens include polycyclic hydrocarbons, phenol compounds derived from coal, among other substances. These substances also interfere with iodide metabolism.

That some natural foods are goitrogenic was evidenced many years ago when it was discovered that rabbits fed a fresh cabbage diet developed goiters that could be reversed by iodine supplementation. It was later shown that vegetables of the cabbage family contained, along with small quantities of thiocyanates, a potent goitrogen that later became known as goitrin (Figure 12.21). Numerous edible plants contain goitrin, including cabbage, kale, cauliflower, broccoli, rutabaga, turnips, Brussels sprouts, and mustard greens. However, these foods are unlikely to be consumed in sufficient quantity to implicate them in the etiology of endemic goiter. Perhaps the only food to be identified directly with goiter etiology is cassava, which is consumed in large quantities in Third World countries. Cassava contains linamarin, a thioglycoside. The linamarin, once hydrolyzed, releases cyanide, which is then metabolized to thiocyanate. Thiocyanate prevents uptake of iodine by the thyroid gland.

EXCRETION

The kidneys have no mechanism to conserve iodide, and they therefore provide the major route (~80%–90%) for iodide excretion [12,13]. The urinary output of iodide correlates closely with both the plasma iodide concentration

and diet such that daily urinary iodine can be used to calculate iodine intake using the following formula: Daily iodine intake = Urinary iodine \times 0.0235 \times body weight, with urinary iodine measured in $\mu\text{g/L}$ and weight measured in kg [14].

Fecal excretion of iodide (up to 20% of the total excreted) is relatively low, ranging from 6.7 to 42.1 $\mu\text{g/day}$ [12]. Some iodide is also lost in sweat, a loss that can be of consequence in hot, tropical regions where iodide intake is marginally adequate.

RECOMMENDED DIETARY ALLOWANCE

Because of its important link to thyroid function, iodide nutriture has been investigated thoroughly for over half a century. Dating as far back as the 1930s, intake requirements have been published based on results of balance studies and on calculations of average daily urinary losses. Adult daily requirements established by those early studies ranged from 100 to 200 μg . The minimum amount (requirement) of iodide to prevent goiter is estimated to be between 50 and 75 $\mu\text{g/day}$ or ~1 $\mu\text{g/kg}$ of body weight.

The intake estimates have not changed significantly over the years. Both the 1989 and 2001 RDA for iodine is 150 $\mu\text{g/day}$ for adults of both sexes and provides a margin of safety to allow for unquantified levels of goitrogens in the diet [2,14]. Although the recommendations apply equally to both sexes, iodide needs are higher during pregnancy and lactation: 220 μg and 290 μg , respectively [14]. The inside cover of the book provides additional RDAs for iodine for other age groups.

DEFICIENCY

Thyroid Hormone Release as Related to Iodide Deficiency

The release of thyroid hormones by the thyroid gland is controlled. Thyrotropin-releasing hormone released from the hypothalamus acts on the pituitary gland to stimulate thyroid-stimulating hormone (TSH). TSH, in response to thyrotropin-releasing hormone, is secreted from the anterior pituitary and increases the activity of the thyroid gland to generate T_4 . TSH output is regulated by T_4 through negative feedback to the pituitary. A decline in the blood level of T_4 triggers release of pituitary TSH, resulting in hyperplasia of the thyroid. Elevated T_4 inhibits release of TSH and thyrotropin-releasing hormone.

Iodine Deficiency and Iodine Deficiency Disorders

Iodine deficiency prevails in many areas of the world and is associated most often with dietary insufficiency of iodine, although deficiencies of other nutrients such as iron, vitamin A, and selenium, also negatively affect the thyroid [15]. Iodine deficiency is the main cause of goiter (although other factors, such as ingestion of goitrogens, may cause

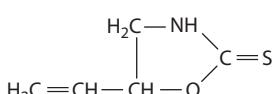


Figure 12.21 Goitrin.

the disorder). Simple goiter is associated most often with inadequate dietary iodine and is characterized by enlargement of the thyroid gland. The enlargement is caused by overstimulation by TSH. Iodide deficiency causes depletion of thyroid iodine stores and therefore reduced output of T_4 and T_3 . As stated above, the decline in the blood level of T_4 triggers release of pituitary TSH, resulting in hyperplasia of the thyroid gland. The growth of the gland is self-restricting, however, because in its enlarged state it traps and processes available iodide more efficiently. The gland returns to normal size over time (months to years) as dietary iodide is increased to adequate amounts. When the prevalence of goiter in any population exceeds 10%, it is called endemic goiter [16].

Because of the effects of iodide deficiency on growth, development, and other health factors, the term iodide deficiency disorders (IDDs) has been implemented. Iodine deficiency in a fetus results from iodide deficiency of the mother, and two types of cretinism can result. Neurological cretinism in the infant is characterized by mental deficiency, hearing loss or deaf mutism, and motor disorders such as spasticity and muscular rigidity [6,16]. Hypothyroid cretinism results in thyroid failure. Early treatment of cretinism with iodine can often correct the condition.

The addition of iodide to table salt and the administration of iodized oil, potassium iodide, or iodine and iron salts have done much to alleviate the problem of endemic goiter in some goitrous regions of the world [15,17]. Yet iodide deficiency continues to be a major health problem in many underdeveloped countries, and, in many countries, may be coupled with selenium and iron deficiencies. See Ma [18] and Carpenter [19] for a review of the history of iodine deficiency.

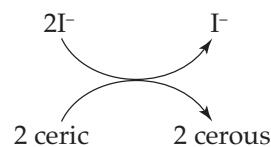
TOXICITY

Excessive iodine intake is reportedly occurring because of poor monitoring and higher than necessary supplementation in several countries with supplementation programs. In addition, in some countries, excessive intake occurs from overconsumption of foods naturally high in iodine. The lowest observed adverse effect level (LOAEL) occurs at iodide intake of ~1,700 $\mu\text{g}/\text{day}$. Some signs of acute iodide toxicity include burning of the mouth, throat, and stomach; nausea; vomiting; diarrhea; and fever. A tolerable upper intake level for iodine has been set at 1,100 $\mu\text{g}/\text{day}$ in response to changes in serum thyrotropin concentrations from varying iodine intake. High iodine intake may cause problems with the thyroid gland, including both hyper- and hypothyroidism and inflammation of the thyroid (thyroiditis). As dietary iodine intake increases, urinary iodine concentrations also rise. Urinary iodine concentrations equal to or in excess of 500 $\mu\text{g}/\text{L}$ have been associated

with increasing thyroid volume, which in turn indicates thyroid dysfunction [20].

ASSESSMENT OF NUTRITION

Iodide nutritional status assessment is generally directed at populations living in areas suspected of being iodide-deficient, although individuals may be assessed if thyroid problems are suspected. Several methods are used for iodine assessment. Urinary iodine excretion represents an indicator of recent iodine intake. The chemistry of tests measuring urinary iodide excretion is based on the ability of the iodide ion to reduce ceric ion (Ce^{4+}), which is yellow, to its colorless, cerous state (Ce^{3+}), as shown here:



The extent of the color change, which is directly proportional to the iodide concentration in the specimen, is monitored spectrophotometrically. All iodine in the specimen, therefore, must first be reduced to iodide. Median urinary iodine concentrations of <100 $\mu\text{g}/\text{L}$ suggests inadequate iodine intake and deficiency in a population.

Thyroid size, measured by ultrasonography or by palpation, is also used to assess iodine status. Enlargement of the gland is associated with suboptimal status; however, the size of the gland may take months to years to return to normal in response to treatment (iodine supplementation) [20]. Thus, the indicator is typically used along with urinary iodine excretion.

Radioactive iodide (^{131}I) uptake may also be measured to assess iodine status. The greater the overall uptake and the quicker the uptake of the radioactive iodide by the thyroid gland, the greater the likelihood of iodide deficiency. In addition, serum TSH concentrations are an especially sensitive indicator of iodine status in newborn infants from at-risk populations. Serum TSH concentrations >5 $\mu\text{-Units}/\text{L}$ in a population suggest deficiency. Further, serum thyroglobulin concentrations >10 $\mu\text{g}/\text{L}$ also suggests inadequate iodine intake.

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Manganese

Although widely distributed in nature, manganese occurs in only trace amounts in animal tissues. The body of a healthy 70 kg man is estimated to contain a total of 10 to 20 mg of the metal. In the body, manganese typically exists in either of two states, Mn^{2+} or Mn^{3+} .

SOURCES

Whole-grain cereals, dried fruits, nuts, and leafy vegetables are among the common manganese-rich foods. Tea also contains large amounts of manganese, but manganese in tea is not well absorbed. The wide content range of the mineral in cereal grains is due partly to plant species differences and partly to the efficiency with which the milling process separates the manganese-rich and manganese-poor parts of the grain. White flour, for example, has a much lower manganese concentration than the wheat grain from which it was produced. Table 12.6 lists the manganese content of selected foods. Usual intake of manganese by Americans ranges from about 1.6 to 2.3 mg/day.

Table 12.6 Manganese Content of Selected Foods and Beverages

Foods/Food Group	Manganese Content (mg/100g)
Bread, whole grains	0.50–2.05
Flour, whole grain	3.80
Bread, white	0.05
Flour, white	0.79
Legumes	0.24–0.58
Nuts	0.83–4.71
Root vegetables	0.05–0.62
Other vegetables	0.15–1.94
Fruits	0.04–1.60
Fruits (dried)	0.09–0.39
Milk and cheeses	<0.01
Beer	0.01
Wine	
White	0.46
Red	0.60
Coffee (brewed)	0.02–0.03
Tea (brewed)	0.18–0.22

Source: www.nal.usda.gov/fnic/foodcomp.

ABSORPTION, TRANSPORT, AND STORAGE

Absorption

Little information is available on the mechanism of manganese absorption, although it has been established that the process occurs equally well throughout the length of the small intestine [1]. Dietary manganese absorption varies considerably, with values of 1% to 14% reported, but absorption is often <5% [2–6]. Gender differences also have been reported, with women absorbing more manganese than men [2]. Manganese absorption from MnCl_2 (manganese chloride) has been shown to be greater than that from plant foods such as lettuce, spinach, and sunflower seeds [3].

The absorption process itself appears to be quickly saturable and probably involves a low-capacity, high-affinity, active transport mechanism such as divalent mineral transporter (DMT)1. With excessive high manganese intake, absorption decreases to protect against toxicity; excretion also increases, as described in the section on manganese excretion. Manganese is thought to be absorbed in the Mn^{2+} state. Within the duodenum, ingested manganese as Mn^{2+} may be converted to Mn^{3+} .

Factors Influencing Absorption Relative to many of the other trace minerals, little information is available on factors influencing manganese absorption. Evidence exists that absorption is enhanced by low-molecular-weight ligands, such as histidine and citrate [7].

Studies in animals and humans suggest that fiber, phytate, and oxalate may precipitate manganese in the gastrointestinal tract, making the manganese unavailable for absorption [7,8]. Several minerals also inhibit manganese absorption. Iron, for example, competes with manganese

for absorption using DMT1. Large amounts of dietary nonheme iron depresses manganese absorption and status, and iron deficiency enhances manganese absorption and retention [9–11]. Negative correlations between serum ferritin and manganese absorption and retention also have been shown [5]. Copper also decreases manganese absorption and retention, while ascorbic acid diminishes Mn-dependent superoxide dismutase activity in rats [11].

Transport and Storage

Manganese entering into the portal circulation from the gastrointestinal tract may either remain free or become bound as Mn^{2+} to α -2 macroglobulin before traversing the liver, where it is almost totally removed. From the liver, some manganese may remain free (Mn^{2+}), or as Mn^{2+} it may be bound to albumin, α -2 macroglobulin, β -globulin, or γ -globulin, or Mn^{2+} may be oxidized by ceruloplasmin to Mn^{3+} and may complex with transferrin [12]. Mn^{3+} bound to transferrin is taken up by transferrin receptors into extrahepatic tissues, including the brain.

Manganese is cleared rapidly from the blood and accumulates preferentially in the mitochondria of tissues, a process that may be mediated by a Ca^{2+} carrier [13]. Within the mitochondria, manganese is present as hydrate Mn^{2+} or Mn^{3+} and as $Mn^{3+}(PO_4)_2$, a matrix precipitate [14]. Manganese is found in most organs and tissues and does not tend to concentrate significantly in any particular one, although its concentration is highest in bone, liver, pancreas, and kidneys. In bone, manganese is found as part of the apatite. Hair also can accumulate manganese.

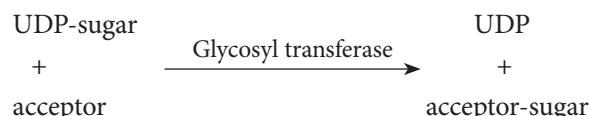
FUNCTIONS AND MECHANISMS OF ACTION

At the molecular level, manganese, like other trace elements, can function both as an enzyme activator and as a constituent of metalloenzymes, but the relationship of these functions to the gross physiological changes observed in manganese deficiency is not well correlated.

In the activation of enzyme-catalyzed reactions, manganese may bind to the substrate (such as ATP) or to the enzyme directly, inducing conformational changes. Enzymes from nearly every class can be activated by manganese in this manner and are numerous and diverse in function. They include enzymes from these enzyme classes: transferases (including kinases), hydrolases, oxidoreductases, ligases, and lyases. The activity of most of these enzymes is not affected by a manganese deficiency, however, largely because the activation is not manganese specific. The metal can be replaced by other divalent cations, primarily magnesium. One exception to this apparent lack of specificity is the manganese-specific activation of the glycosyl transferases. Examples of some manganese-dependent enzymes from each enzyme class are described in the next section.

Transferases

Many transferases require manganese. Two examples are xylosyl transferases and glycosyl (or called galactosyl) transferases. Glycosyl transferases catalyze the transfer of a sugar moiety such as galactose from uridine diphosphate (UDP) to an acceptor molecule, as shown by the general reaction:



Several sugars participate in these reactions. Galactose is a more common participant and when bound to UDP may be transferred to an acceptor molecule by the glycosyl transferase. Glycosyl transferases are necessary for proteoglycan, including mucopolysaccharide, synthesis. Remember that mucopolysaccharides, among other proteoglycans, are important components of bone and connective tissue such as collagen.

Hydrolases

Manganese also activates prolidase, a dipeptidase with specificity for dipeptides. Prolidase is found in dermal fibroblasts and catalyzes the final step in collagen degradation. Arginase, which requires four manganese atoms per molecule, is a cytosolic enzyme responsible for urea formation and found in high concentrations in the liver. The Mn^{2+} may allosterically activate arginase through a pH-mediated role [15]. Low manganese diets in animals have been shown to decrease arginase activity [16].

Lyases

Phosphoenolpyruvate carboxykinase (PEPCK), also activated by manganese, converts oxaloacetate to phosphoenolpyruvate and carbon dioxide. This reaction is important in gluconeogenesis. The activity of phosphoenolpyruvate carboxykinase decreases in animals with manganese deficiency.

Oxido-Reductases

Superoxide dismutase, a manganese-dependent (Mn^{3+} -SOD) metalloenzyme (not manganese activated), functions in a manner similar to copper- and zinc-dependent superoxide dismutase to prevent lipid peroxidation by superoxide radicals. Manganese SOD is found in the mitochondria, however, whereas copper-zinc SOD is found both extracellularly and in the cytoplasm. Thus, SOD in the mitochondria likely eliminates superoxides before they damage mitochondrial function. The activity of the electron transport/respiratory chain generates large amounts of superoxide radicals, necessitating substantial Mn-SOD activity. The cell ultrastructural abnormalities associated with manganese deficiency are likely caused by unchecked lipid peroxidation in the cellular

membranes because of reduced Mn-SOD activity or simply by reduced availability of manganese to directly scavenge free radicals. Manganese (Mn^{2+}), one of several minerals able to scavenge free radicals, quenches peroxy radicals as shown in this equation [17]: $Mn^{2+} + ROO^{\bullet} \longrightarrow Mn^{3+} + ROOH$. Low-manganese diets in animals have been shown to decrease Mn-SOD activity.

Ligases/Synthetases

Pyruvate carboxylase, which contains four manganese atoms, converts pyruvate to oxaloacetate, a TCA cycle intermediate. Because magnesium can replace manganese in pyruvate carboxylase, minimal changes in pyruvate carboxylase activity occur [16]. Glutamine synthetase may be a manganese metalloenzyme or may be activated by manganese or magnesium.

Other Roles

Manganese also may act as a modulator of second messenger pathways in tissues. For example, manganese increases cAMP accumulation through binding to ATP and ADP. Manganese can activate guanylate cyclase, and manganese may affect cytoplasmic calcium levels and thus regulate calcium-dependent processes [14].

INTERACTIONS WITH OTHER NUTRIENTS

Only a few interactions between manganese and other trace elements are thought to be of significance nutritionally. One relationship of nutritional significance—that between manganese and iron—is detailed in the section on absorption. However, the interaction is reciprocal: that is, iron in excess inhibits manganese absorption, and manganese, when ingested in amounts about four to eight times recommended intake, decreases iron absorption up to about 40%. Some degree of interaction may occur between manganese and calcium and between manganese and zinc in such a way as to affect the bioavailability of manganese. However, because of the paucity of information and the divergent results of some relevant studies, the nature of such interactions remains inconclusive.

EXCRETION

Manganese is excreted primarily (>90%) via the bile in the feces. Excess absorbed manganese from the diet is quickly excreted by the liver into the bile to maintain homeostasis [2]. Very little manganese is excreted in the urine. Moreover, urinary manganese does not correlate with intake and does not increase even when dietary intake of the mineral is excessive [18,19]. However, excretion of manganese through sweat and skin desquamation has been shown to contribute to manganese losses [20].

ADEQUATE INTAKE

In 1980, the Food and Nutrition Board first recommended an estimated safe and adequate daily dietary intake for manganese of 2.5 to 5 mg for adults, and in 1989 this range was modified to 2 to 5 mg [21]. This recommendation was thought to represent a dietary intake level achieved by most people who exhibit no signs of deficiency or toxicity [21]. The 2001 recommendation, like previous recommendations, is based on median intake, because data are insufficient to calculate the requirement for manganese. The latest recommended intakes are 2.3 mg for adult men and 1.8 mg for adult women [22]. With pregnancy and lactation, recommendations increase to 2 mg and 2.6 mg, respectively [22]. The inside cover of the book gives additional recommendations for manganese for other age groups.

DEFICIENCY

Studies on a wide variety of species have demonstrated that manganese deficiency is associated with striking and diverse physiological malfunctions. Manganese deficiency generally does not develop in humans unless the mineral is deliberately eliminated from the diet. Studies in which men received either 0.11 mg manganese per day for 39 days (the diet was also devoid of vitamin K, making it difficult to separate the effects of the manganese and vitamin K deficiencies) or 0.35 mg manganese per day resulted in negative manganese balance [20,21]. Symptoms and signs of deficiency included nausea; vomiting; dermatitis; decreased serum manganese; decreased fecal manganese excretion; increased serum calcium, phosphorus, and alkaline phosphatase (thought to be associated with skeletal bone changes); decreased growth of hair and nails; changes in hair and beard color; poor bone formation and skeletal defects; and altered carbohydrate and lipid metabolism [20–23]. Other effects reported included the occurrence of neonatal ataxia and loss of equilibrium, cell ultrastructure abnormalities, compromised reproductive function, abnormal glucose tolerance, and impaired lipid metabolism [20–22]. In rats, dietary manganese deficiency also altered plasma ammonia and urea concentrations in association with decreased arginase activity.

TOXICITY

Manganese toxicity can occur in people with liver failure, because manganese homeostasis is maintained through bile excretion. Manganese toxicity secondary to liver failure is characterized by manganese accumulation within the liver and other organs such as the brain; accumulation in the brain results in neurologic abnormalities [24,25]. Neonates receiving total parenteral nutrition are thought to be at risk for manganese toxicity because of lack of

absorptive control and diminished excretion [26,27]. Miners who have inhaled dust fumes high in manganese (about 5 mg/m³ or more) experience Parkinsonism-like symptoms. Manganese toxicity in people chronically exposed to airborne manganese in concentrations as low as 1 mg/m³ also have been reported to experience problems, including prolonged reaction time, tremors, and diminished memory capacity [28]. The tolerable upper intake level for manganese has been set at 11 mg/day [22].

ASSESSMENT OF NUTRITION

Assessment of manganese status typically is based on concentrations of manganese in mononuclear blood cells as well as in plasma, serum, and whole blood [29]. Serum concentrations have been found to be somewhat sensitive to large variations in intake but do not necessarily correlate with intake [19]. It has been suggested that mononuclear blood cell concentrations of manganese may be a better indicator than blood or serum concentrations [29]. Enzyme activity also has been used to assess status. In animals, mitochondrial Mn-SOD in some tissues and blood arginase activities have been shown to be diminished with low manganese intake or deficiency [9,11,30]. In humans, manganese supplementation significantly increased lymphocyte Mn-SOD activity and serum manganese concentrations from baseline without changes in manganese excretion [18]. Additional laboratory tests to assess body manganese status are still under investigation.

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Molybdenum

The need for molybdenum was established in humans through the observation that a genetic deficiency of specific enzymes that require molybdenum as a cofactor resulted in severe pathology. In the body, molybdenum, a metal, is found primarily in either of two valence states,

Mo^{4+} or Mo^{6+} . In biological systems, molybdenum generally is bound to either sulfur or oxygen.

SOURCES

Molybdenum is widespread among foods, but as with many other minerals, the molybdenum content of a given plant food may vary greatly depending on the concentration of molybdenum in the soil. It follows that the metal's content in meats in turn reflects its concentration in the regional forage. Better sources of molybdenum in the diet are legumes, which can provide up to 184 $\mu\text{g}/100 \text{ g}$; meat, fish, and poultry, which contain up to $\sim 129 \mu\text{g}/100 \text{ g}$; and grains and grain products, which provide up to $\sim 117 \mu\text{g}/100 \text{ g}$ [1,2]. Nuts and vegetables usually contain $<50 \mu\text{g}/100 \text{ g}$, but fruits and dairy products are especially low in molybdenum, providing $<12 \mu\text{g}/100 \text{ g}$ [1,2].

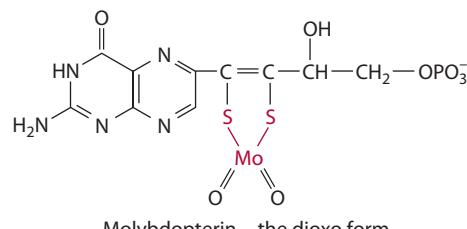
ABSORPTION, TRANSPORT, AND STORAGE

Little is known about the sites from which molybdenum is absorbed in humans. The mechanism of absorption is thought to be passive, although some animal studies suggest the possible involvement of carriers. Absorption increases with increasing dietary intake over a range of 22 to 1,490 $\mu\text{g}/\text{day}$ [3]. Absorption in humans ranges from $\sim 50\%$ to over 90% [4–6]. Transport of molybdenum in the blood is thought to occur as molybdate (MoO_4^{2-}). The mineral may be bound to albumin or to α -2 macroglobulin.

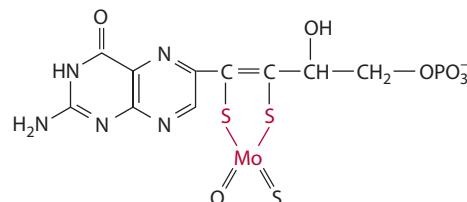
The molybdenum content of human tissues is quite low under normal dietary conditions, averaging 0.1 to 1.0 $\mu\text{g/g}$ of wet weight. Molybdenum is found in tissues as molybdate, free molybdopterin, or molybdopterin that is bound to enzymes. The liver, kidneys, and bone contain the most molybdenum in terms of both absolute amount and concentration [7]. Other tissues, such as the small intestine, lungs, spleen, brain, thyroid and adrenal glands, and muscle, also contain molybdenum.

FUNCTIONS AND MECHANISMS OF ACTION

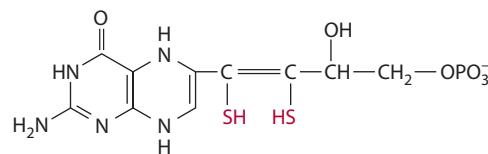
The biochemical role of molybdenum centers around the redox function of the element and its necessity as a cofactor in the form of molybdopterin for three metalloenzymes (sulfite oxidase, aldehyde oxidase, and xanthine dehydrogenase/oxidase), all of which catalyze oxidation-reduction reactions [8]. Molybdopterin is an alkylphosphate-substituted pterin, to which molybdenum is coordinated through two sulfur atoms [9–11]. Molybdopterin anchors the molybdenum to the apoenzyme at its catalytic site. The molybdenum is further bonded either to two oxygen molecules (called dioxo-molybdopterin) or to one oxygen and one sulfur (called oxosulfidomolybdopterin), as shown in Figure 12.22.



Molybdopterin—the dioxo form



Molybdopterin—the oxosulfido form



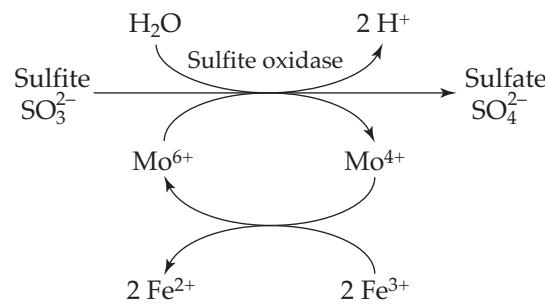
Pterin—without the molybdenum attached

Figure 12.22 Molybdopterin structures.

The inability to synthesize molybdopterin because of genetic defects is usually lethal.

Sulfite Oxidase

Sulfite oxidase, a mitochondrial intermembrane enzyme found in many body tissues, especially the liver, heart, and kidney, has iron-sulfur clusters and two molybdopterin (dioxo cofactor form) and two cytochrome residues. The enzyme catalyzes the terminal step in the metabolism of sulfur-containing amino acids (methionine and cysteine), in which sulfite (SO_3^{2-}) is converted to sulfate (SO_4^{2-}), as shown here:



Sulfites also may originate from the diet, because they are added to some foods as an antimicrobial agent. Cytochrome c is the physiological electron acceptor for the reaction. Sulfate generated from this reaction typically is excreted in the urine or reused for the synthesis of sulfoproteins, sulfolipids, and mucopolysaccharides (a component of mucus).

Aldehyde Oxidase

Aldehyde oxidase is a molybdoenzyme (using the oxosulfido form) that is very similar to xanthine oxidase (see the next section) in size, cofactor composition, and substrate specificity. It presumably functions in the liver as a true oxidase, using molecular oxygen as its physiological electron acceptor. The enzyme's primary substrates are thought to include a variety of aldehydes, including drugs [12]. Other enzymes, however, such as an NADH-dependent aldehyde dehydrogenase also found in the liver, are thought to catalyze reactions similar to those catalyzed by this aldehyde oxidase.

Xanthine Dehydrogenase and Xanthine Oxidase

Xanthine dehydrogenase and xanthine oxidase (also called oxidoreductases) are iron-dependent, (iron-sulfur centers nonheme-containing) enzymes that also require FAD and molybdopterin in the oxosulfido cofactor form. Xanthine dehydrogenase is found in a variety of tissues, including the liver, lungs, kidneys, and intestine. Xanthine oxidase is found in the intestine, thyroid cells, and possibly other tissues. Healthy tissues may contain about 10% of their total xanthine enzymes in the oxidase form [13]. Conversion of xanthine dehydrogenase to xanthine oxidase may occur following oxidation of essential sulfhydryl groups or by proteolysis of the dehydrogenase form.

The xanthine dehydrogenase and oxidase enzymes are capable of hydroxylating various purines, pteridines, pyrimidines, and other heterocyclic nitrogen-containing compounds. Hypoxanthine, derived from purine catabolism, is oxidized in most tissues by xanthine dehydrogenase to generate xanthine and then uric acid (Figure 12.23). Xanthine dehydrogenase transfers electrons from the substrate onto NAD^+ to form $\text{NADH} + \text{H}^+$. Oxidation of hypoxanthine and xanthine by xanthine oxidase also results in uric acid, but in these reactions O_2 accepts the electrons from FADH_2 and hydrogen peroxide (H_2O_2) or a superoxide radical is formed.

Although low-molybdenum diets or those that include tungstate, a molybdenum antagonist, predictably reduce the level of xanthine oxidase activity in rat intestine and liver, no apparent clinical effects result from the perturbation. Furthermore, the human inheritable disorder xanthinuria, in which large amounts of xanthine are excreted in the urine, provides additional evidence of the body's ability to tolerate low xanthine dehydrogenase or oxidase activity. The condition is essentially free of clinical manifestations, except for the possible development of kidney calculi (stones) caused by the high urinary xanthine concentration. Therefore, whether any of the reactions catalyzed by xanthine dehydrogenase or oxidase are necessary for human health is not firmly established [14].

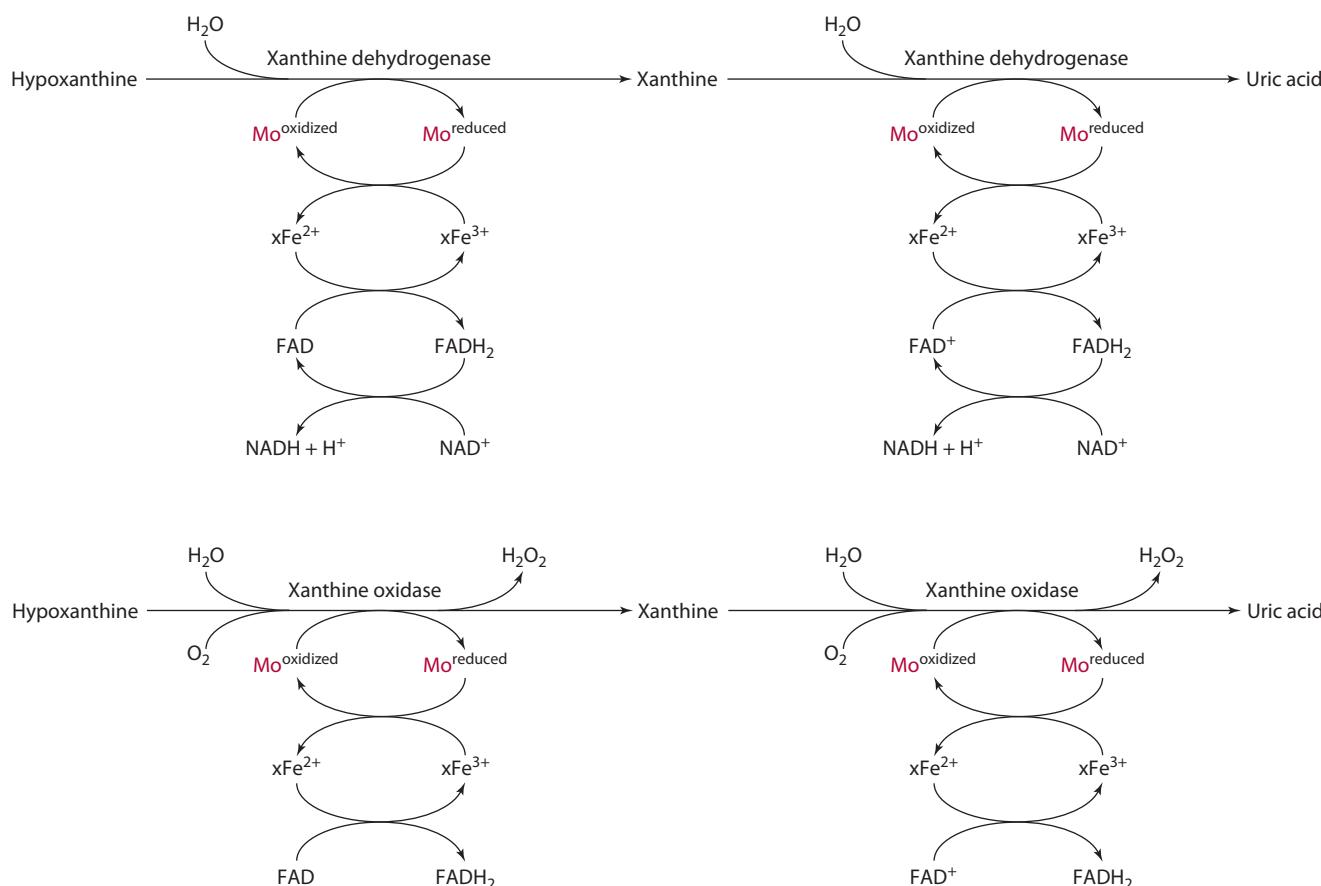


Figure 12.23 The actions of xanthine dehydrogenase and xanthine oxidase on the substrate hypoxanthine.

The effects of xanthine oxidase activity, however, are quite damaging in people being treated for ischemia (local or temporary deficiency of blood supply and thus relative oxygen deprivation), for example. Degradation of ATP in hypoxic tissue yields hypoxanthine. Reperfusion of the intestine with oxygen (as occurs with medical treatment of intestinal ischemia, for example) helps prevent total destruction of the tissue from lack of oxygen and nutrients, but it also provides xanthine oxidase, with the oxygen needed to oxidize the relatively large concentrations of hypoxanthine. Oxidation of hypoxanthine generates large amounts of hydrogen peroxide, which further induces tissue damage (called reperfusion injury). However, the injury also is thought to be mediated partly by neutrophil accumulation and activation, and the reactive oxygen species formed may be involved in signal transduction pathways [15].

Another role for molybdenum, in addition to its biochemical role, may involve modulation (likely inhibition through direct interaction) of the glucocorticoid receptor complex [16].

INTERACTIONS WITH OTHER NUTRIENTS

Tungsten has long been recognized as a potent antagonist of molybdenum [17], and in fact its administration into test animals has become the major means for artificially creating a state of molybdenum deficiency.

Another interaction involves molybdenum, sulfur, and copper. It has been shown, particularly in ruminants, that a high dietary intake of sulfate or molybdenum depressed the tissue uptake of copper and, conversely, that sulfate and copper decreased molybdenum retention [18]. The proposed explanation for this interaction is that sulfide and hydrosulfide ions are generated in the rumen by reduction of ingested sulfate. The reactive sulfide then displaces oxygen from molybdate ions, yielding oxythiomolybdates and tetrathiomolybdates. Molybdenum is not readily absorbed in the form of thiomolybdates, and, furthermore, thiomolybdates bind copper avidly, rendering that metal less physiologically available [19]. Note, however, that in humans and other nonruminants such an interaction is not as important, because of the low yield of sulfides and hydrosulfides resulting from sulfate reduction during digestion. However, feeding tetrathiomolybdates to nonruminant test animals does result in a compromised uptake of copper. Therefore, the antagonistic effect of molybdenum or sulfate on copper availability appears to be attributable to the tendency of molybdenum to sequester reactive sulfide groups. These groups subsequently bind copper ions, which then become less available.

A relationship between molybdenum intake and copper excretion has been documented in one study in humans. Urinary copper excretion in humans has been shown to rise from 24 µg/day to 77 µg/day as molybdenum intake

increased from 160 µg to 1,540 µg/day [20]. No changes in fecal copper excretion were noted, suggesting perhaps that molybdenum increased copper mobilization from tissues and promoted excretion [20]. These effects were not confirmed, however, in a later study by Turnlund and Keys [21].

Other nutrients and substances that appear to affect molybdenum availability, by mechanisms not yet understood, include manganese, zinc, iron, lead, ascorbic acid, methionine, cysteine, and protein. A possible relationship with silicon is described in Chapter 13's section on silicon.

EXCRETION

Most molybdenum is excreted as molybdate from the body in the urine. Furthermore, urinary excretion of molybdenum increases as dietary molybdenum intake increases [5,6]. In other words, little molybdenum is retained in the body when dietary intake is high, and the kidney is thought to play a role in molybdenum homeostasis [3,5,6]. Small amounts of molybdenum are excreted from the body in the feces by way of the bile [5]; small amounts also can be lost in sweat (20 µg) and in hair (0.01 µg/g hair).

RECOMMENDED DIETARY ALLOWANCE

In 1980, an estimated safe and adequate daily dietary intake range for molybdenum for adults of 150 to 500 µg was recommended. In 1989, this range was adjusted to 75 to 250 µg [22]. In the last decade, balance as well as depletion and repletion studies have suggested a minimum requirement of 25 µg molybdenum per day [5,6]. Based on 75% absorption, an estimated average requirement for adults was set at 34 µg [23]. The 2001 RDA for molybdenum for adults (men and women) is 45 µg (130% of the requirement), with 50 µg suggested during pregnancy and lactation [23]. The inside cover of the book provides additional RDAs for molybdenum for other age groups.

DEFICIENCY

Molybdenum deficiency is rarely encountered unless the diet is particularly rich in antagonistic substances such as sulfate, copper, or tungstate. Low molybdenum intakes have been associated with esophageal cancer in China. Molybdenum deficiency has been documented in a patient maintained on total parenteral nutrition for 18 months [24]. The patient exhibited high blood concentrations of methionine, hypoxanthine, and xanthine, as well as low blood levels of uric acid. Urinary concentrations of sulfate were low, and those of sulfite were high. Treatment with 300 µg ammonium molybdate (163 µg molybdenum) resulted in clinical improvement and normalized sulfur amino acid metabolism and uric acid production.

The importance of sulfite oxidase, and therefore molybdenum, in human nutrition is evidenced by the neurological disorders associated with a genetic deficiency of sulfite oxidase in children [25]. Elevated levels of urinary sulfite and thiosulfate, along with biochemical manifestations reflecting aberrant sulfur amino acid metabolism and sulfite oxidation, were observed.

TOXICITY

Molybdenum appears to be relatively nontoxic, with intake up to 1,500 µg/day [5]. However, symptoms such as gout (inflammation of the joints caused by accumulation of uric acid) have appeared in some people living in regions that contain high soil molybdenum levels and in those with occupational exposure to molybdenum [26]. Gout results from high uric acid concentrations (which likely have arisen from increased xanthine dehydrogenase activity) that have accumulated in and around joints. A tolerable upper intake level for molybdenum has been set at 2 mg [23].

ASSESSMENT OF NUTRITION

Molybdenum appears to distribute itself fairly equally between the plasma and red blood cells. Although a few studies have reported the molybdenum concentrations of human plasma and blood, the use of these as indicators of molybdenum status has not been validated.

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Fluoride

Whereas fluorine (F) is a gaseous chemical element, fluoride (F⁻) exists and is composed of fluorine bound to a metal, nonmetal, or organic compound. Fluoride is found in nature and in the human body in trace amounts. The term *fluoride* is used throughout this section. Analogous to this terminology is the use of the terms *iodide* and *chloride*. Fluoride is not considered an essential nutrient, but it is clearly recognized as important for the health of bones and teeth, as detailed under the section “Functions and Mechanisms of Action.”

SOURCES

Community drinking water has been fluoridated (1 ppm or 1 mg/L) for nearly 50 years in the United States following the discovery in 1942 of the inverse relationship between fluoride intake and the incidence of dental caries [1]. This discovery has in turn affected the distribution of fluoride in foods and beverages. Several beverages, including

Table 12.7 Fluoride Content of Various Food Groups

Food Group	Fluoride Content Range (mg/100g)
Dairy products	0.002–0.082
Meat, poultry	0.004–0.092
Grain, cereal products	0.008–0.201
Potatoes	0.008–0.084
Green leafy vegetables	0.008–0.070
Legumes	0.015–0.057
Root vegetables	0.009–0.048
Other vegetables, vegetable products	0.006–0.017
Fruits	0.002–0.013
Fats, oils	0.002–0.044
Sugar adjuncts	0.002–0.078

Source: From Donald R. Taves, "Dietary intake of fluoride ashed (total fluoride) v. unashed (inorganic fluoride) analysis of individual foods" in the *British Journal of Nutrition*, Vol. 49, Issue 03, May 1983, pp. 295–301. Reprinted with the permission of Cambridge University Press.

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ready-to-use infant formulas, are made with fluoridated water. Other beverages vary greatly in their fluoride content depending on the use or nonuse of fluoridated water in their processing.

The fluoride content in most food groups is low, usually <~0.05 mg/100 g [2]. However, a few foods contain higher amounts of fluoride, including some grains and cereal products, some marine fish (if consumed with the bones), and tea. Marine fish contain from ~0.01 to 0.17 mg/100 g [2]. Tea, both caffeinated and decaffeinated, is rich in F[−] because tea leaves accumulate the element in fairly high amounts. Brewed tea contains from 1 to 6 mg/L, with decaffeinated forms higher in fluoride than caffeinated [3,4]. Table 12.7 provides the fluoride content of various food groups. Most Americans obtain most dietary fluoride from drinking water, which provides about 0.24 mg fluoride/cup. Usual fluoride intake by Americans is up to about 3.4 mg per day. However, swallowing fluoride-containing toothpaste can be a significant source of the mineral. Such practices can be dangerous, especially to young children, because ingesting even small amounts of fluoride-containing toothpaste can result in ingestion of fluoride in amounts exceeding recommended intake. Consequently, many toothpaste manufacturers recommend that toothpaste be kept out of reach of children and that only a "pea-sized" amount of toothpaste be used for tooth brushing.

DIGESTION, ABSORPTION, TRANSPORT, AND STORAGE

Absorption of fluoride is nearly 100% when it is consumed as sodium fluorosilicate in fluoridated water or as sodium fluoride or monofluorophosphate in toothpaste. Absorption diminishes to ~50%–80% when fluoride is consumed with solid foods or with calcium-containing beverages. Calcium, along with other minerals (both di-

trivalent), is thought to form insoluble complexes with fluoride to decrease its absorption. In foods, fluoride also may be found bound to proteins. Protein-bound fluoride must be hydrolyzed by gastric pepsin or other intestinal proteases before absorption, and less fluoride is absorbed from these sources than from water or toothpaste. Fluoride is relatively poorly absorbed (37%–54%) from foods such as bone meal [5].

Humans, given small amounts of soluble fluoride, achieve maximum blood fluoride levels within ~30 to 90 minutes [6]. This rapidity of absorption is accounted for by the fact that it occurs to a great extent in the stomach, a rather unique characteristic among the elements.

Fluoride absorption is believed to occur by passive diffusion. Fluoride's rapid gastric absorption can be explained by the fact that it exists primarily as hydrogen fluoride, also called hydrofluoric acid (HF), at the low pH of the gastric contents rather than as ionic fluoride. The rate of diffusion across membranes, in general, correlates directly with the lipid solubility of the diffusing substance. Gastric diffusion is also inversely related to pH. The pH dependence of gastric absorption is consistent with the hypothesis that HF is being absorbed [6]. Hydrogen fluoride is a weak acid with a pKa (the negative logarithm of an acid dissociation constant, Ka) of 3.4, dissociating according to the equation HF → H⁺ + F[−]. Assuming a gastric pH of ~1.5, the ratio of HF to F[−], readily calculable from the Henderson-Hasselbalch equation as discussed in Chapter 15 under the section on acid-base buffers, would be nearly 200:1 [6]. The gastric luminal form of fluoride is therefore largely diffusible. Fluoride is also absorbed throughout the small intestine, but at a reduced rate. Fluoride absorption does not appear to be influenced by its plasma levels except at a very high, perhaps toxic, concentration [7].

Some fluoride is transported in the blood as ionic fluoride or hydrofluoric acid, not bound to plasma proteins. Some fluoride is strongly bound and is called nonionic or the organic form. Organically bound fluoride occurs in variable concentrations that are independent of total fluoride intake and plasma ionic fluoride levels. What is not known is the extent to which environmental contamination by industrially generated fluorocarbons may contribute to the variability. Ionic fluoride concentration, in contrast, correlates directly with dietary intake even up to very large oral doses, indicating that plasma ionic fluoride is not precisely controlled by homeostatic mechanisms.

Absorbed fluoride leaves the blood very quickly and is distributed rapidly throughout the body. Most fluoride is found in bones and teeth. Fluoride associated with bone is found in both an amorphous state (rapidly exchangeable pool) and a crystalline state (slowly exchangeable pool). In its more crystalline state, fluoride is sequestered in bones by apatite, a basic calcium phosphate with the

theoretic formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Mineralized tissues account for nearly 99% of total body fluoride, with bone being by far the major depot. As the amount of absorbed fluoride increases, so does the quantity taken up by hard tissue. However, the percentage retained at high absorption rates becomes lower because of accelerated urinary excretion [7]. Skeletal growth rate influences fluoride balance, exemplified by the fact that young, growing people incorporate more fluoride into the skeleton than adults and excrete less in the urine.

FUNCTIONS AND MECHANISMS OF ACTION

The major functions of fluoride are related to its effects on the mineralization of teeth and bones. Specifically, it promotes mineral precipitation from amorphous solutions of calcium and phosphate, leading to the formation of apatite, a crystalline structure. The apatite is deposited as crystallites within an organic (protein) matrix.

Fluoride can be incorporated into the apatite structure by replacing hydroxide ions. Ions can be replaced during initial crystal formation or by displacement from previously deposited mineral, according to the following equation: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + x\text{F}^- \longrightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-x}\text{F}_x$. With the deposition of fluoride in the hydroxyapatite in the enamel, fluorohydroxyapatite is formed. This compound has been shown to be less acid-soluble than hydroxyapatite and thus more resistant to cavity formation [8,9]. The extent of fluoride incorporation varies with animal species, age, fluoride exposure, and rate of tissue turnover. In bone and dental enamel of humans and other higher mammals, the ratio of substitution of F^- for OH^- is from ~1:20 to 1:40.

Mineral deposition and stimulation of new bone formation also have been attributed to fluoride [10,11]. Enamel's matrix protein has a high affinity for fluoride, which has led to the speculation that fluoride's major role in mineralization may be its participation in the nucleation of crystal formation rather than its association with the mineral phase [12]. In addition to its role in mineralization, topical fluoride appears to decrease acid production by oral plaque-producing bacteria [11].

INTERACTIONS WITH OTHER NUTRIENTS

It has been reported that aluminum, calcium, magnesium, and chloride reduce fluoride uptake and use, whereas phosphate and sulfate increase its uptake [13]. Sodium chloride, for example, decreases the skeletal uptake of fluoride [14]. This point is of interest because kitchen or table salt has been used as a vehicle for fluoride supplementation in some countries. The mechanisms of the interactions are not established.

Aluminum-containing antacid use also reduces the absorption of fluoride as well as phosphorus. Aluminum is

thought to form insoluble complexes with fluoride in the intestine [15]. The contribution of the pH effect of the antacid on fluoride availability requires further investigation.

EXCRETION

Fluoride is rapidly excreted in the urine, accounting for approximately 90% of total excretion. Some renal tubular reabsorption occurs by passive diffusion of undisassociated HF. Because the amount of HF is increased relative to the amount of F^- as acidity is increased, tubular reabsorption and urinary pH are inversely related [6]. That is, the extent of reabsorption is inversely related to the pH of the fluids in the tubules. Fecal elimination accounts for most of the remaining losses, with only minor losses occurring in sweat.

ADEQUATE INTAKE

The 1997 Dietary Reference Intakes reported adequate intakes of 4 and 3 mg fluoride/day for adult males and females, respectively [16]. No increases are recommended for women during pregnancy or lactation.

Fluoridation of water at 1 to 2 ppm continues to be recommended by the American Dental Association to optimize dental health [17,18]. The inside cover of the book provides additional AIs for fluoride for other age groups.

DEFICIENCY

Fluoride deficiency in test animals has been reported to result in curtailed growth, infertility, and anemia. However, these findings are not well documented and clearly cannot be extrapolated to predict similar effects on humans. In humans, an optimal level of fluoride helps to reduce the incidence of dental caries and perhaps also to maintain the integrity of skeletal tissue.

TOXICITY

Chronic toxicity of fluoride, called fluorosis, is characterized by changes in bone, kidney, and possibly nerve and muscle function [6,18]. Dental fluorosis or mottling of teeth has been observed in children receiving 2 to 8 mg fluoride/kg body weight. Acute toxicity manifests as nausea, vomiting, diarrhea, acidosis, and cardiac arrhythmias. Death has been reported following ingestion of between 5 and 10 g sodium fluoride or ~32 to 64 mg fluoride/kg body weight, although it may occur with an intake as low as 5 mg fluoride/kg body weight [6,18]. The tolerable upper intake levels for fluoride range from 1.3 mg/day for children age 1 to 3 years to 10 mg/day for children older than 8 years and adults [16].

ASSESSMENT OF NUTRITION

Normal ranges for ionic fluoride have been established at 0.01 to 0.2 µg F⁻/mL plasma and 0.2 to 1.1 mg F⁻/mL urine. The element is most commonly determined in its ionic form by fluoride ion-specific electrode potentiometry, a technique analogous to the hydrogen ion-specific electrode potentiometry of the common pH meter.

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Nutrient–Drug Interactions

Nutrient–drug interactions represent nutrient-induced changes in the kinetics of a drug or drug-induced changes in nutrient metabolism or nutritional status. Such interactions can be extremely detrimental. Some nutrient interactions can lead to failure of the drug to perform its desired actions or can lead to drug toxicity. Alternately, some drug interactions can promote nutrient deficiencies or toxicities. In addition to direct interactions, drugs also may influence nutrition status through multiple other mechanisms such as diminishing appetite, altering taste, or promoting nausea, vomiting, or diarrhea. This Perspective reviews some examples of foods or nutrients that affect the absorption, distribution, metabolism, actions (functions), or excretion of drugs, as well as some examples of drugs that affect the absorption, metabolism, or excretion of nutrients. Table 1 provides

an overview of some of the interactions presented in this Perspective.

Effects of Foods/Nutrients on Drug Absorption

Foods or nutrients in foods can alter drug absorption by their presence as a physical barrier or through effects on transit time (i.e., motility of the gastrointestinal tract), secretions, drug dissolution, chelation, or carrier uptake, among other effects. Many drugs should be taken without food or beverage to prevent interference with drug absorption. For example, the absorption of Fosamax (alendronate), used to treat osteoporosis, is greatly diminished with concurrent ingestion of food or beverages (other than water). Similarly, the antibiotics erythromycin and penicillin (ampicillin), along with the antihypertensive drugs Capoten (captopril) and

Univasc or Uniretic (moexipril), should be ingested only with water; food should not be consumed for at least 1 hour. Alternately, the absorption of many other drugs is enhanced with coingestion of food or even specific dietary nutrients such as a high-fat meal.

Foods or antacids that contain relatively high amounts of magnesium, calcium, zinc, iron, and aluminum need to be avoided or should be ingested separately (by several hours) from antibiotics such as Achromycin and Sumycin (tetracycline antibiotics), Cipro (ciprofloxacin), Maxaquin (lomefloxacin), and Levaquin (levofloxacin), and from other groups of antibiotics and antifungals such as Nizoral (ketoconazole). The divalent and trivalent minerals in the antacids or from the foods chelate (bind to) and decrease the absorption of the drugs.

An appropriate gastrointestinal tract pH is important for dissolving or absorbing some drugs. Thus, ingesting foods or antacids that can promote gastrointestinal secretions or alter pH may be detrimental. The antifungal agent ketoconazole, for example, needs an acidic pH to dissolve. Other drugs are damaged in an acidic pH and thus need to be ingested without food or beverage (except water).

Effects of Foods on Drug Metabolism

Many drugs that undergo substantial first pass metabolism in the gastrointestinal tract are affected by coingestion of grapefruit juice, including the immunosuppressants Neoral and Sandimmune (cyclosporine); some HMG CoA reductase inhibitors (used to treat high blood cholesterol) such as Zocor (simvastatin), Mevacor (lovastatin), and Lipitor (atorvastatin); Pletal (cilostazol), which is used to treat intermittent claudication and peripheral vascular disease; VePesid (etoposide) used to treat some cancers; and Relpax (eletriptan) used to treat migraines. References with more comprehensive lists of drugs known to interact with grapefruit juice are listed at the end of this Perspective [1–4]. The exact compound or compounds in grapefruit juice thought to cause the interaction are not clear. Grapefruit juice is rich in many phytochemicals, especially flavonoids such as the flavanone naringenin and its glycoside naringin and the flavonol kaempferol. Ingesting grapefruit juice is thought to decrease (down-regulate) the isozyme of cytochrome P₄₅₀ known as CYP 3A4, which is found in the intestine. This enzyme normally begins intestinal cell

Table 1 An Overview of Some Selected Drug–Nutrient/Food Interactions

Drug(s)	Nutrient(s)/Food(s)
Antibiotics —tetracycline, Achromycin, Sumycin, Cipro, Maxaquin, and Levaquin;	Calcium, magnesium, zinc, iron, and aluminum
Antifungal —Nizoral	
Immunosuppressant —Neoral; some HMG CoA reductase inhibitors —Zocor, Mevacor, and Lipitor; Anti-intermittent claudication —Pletal; Antimigraine —Relpax	Grapefruit juice
Antiparkinson —Dopar, Larodopa, Sinemet, Parcopa	Protein and vitamin B₆
Monoamine oxidase inhibitors —Parnate and Nardil; Antituberculosis —Isoniazid	Amine-containing foods —aged cheeses; smoked, salted, and pickled fish; sausage; salami; pepperoni; corned beef; bologna; meat extracts; wines; and chocolate; among others
Anticoagulants —Coumadin	Vitamin K
Bronchodilators —Theo-24, Theolair, Uniphyll, and Elixophyllin	Caffeine and vitamin B₆
Antimanic —Eskalith, Lithobid, Lithotabs	Sodium
Bile-acid sequestrants —Questran	Fat-soluble vitamins A, D, E, and K; folate; iron; magnesium; calcium; and zinc
Antituberculosis —Isoniazid	Vitamin B₆
Anticonvulsants —Phenobarbital, Dilantin, and Phenytek	Vitamin D and folate
H₂ receptors blockers —Tagament and Zantac; Proton-pump inhibitors —Prilosec and Prevacid	Iron, zinc, calcium, magnesium, aluminum, and vitamin B₁₂
Loop Diuretics —Lasix and Bumex	Potassium, chloride, magnesium, and sodium

metabolism of many drugs. Consequently, the down-regulation of this enzyme by ingestion of grapefruit juice causes the drugs to be absorbed without any metabolism and causes blood concentrations of the drugs to be much higher than desired. The high blood drug concentrations, in turn, can result in undesirable side effects, including toxicity.

In addition to alterations in drug distribution caused by grapefruit juice, a high protein intake (two to three times recommendations) alters the distribution of the anti-Parkinson drugs Dopar and Larodopa (levodopa), and Sinemet and Parcopa (levodopa and carbidopa). The effects are thought to result from competition for carriers at the blood-brain barrier between the drug and large neutral amino acids (such as phenylalanine, tyrosine, and tryptophan). These large neutral amino acids appear in the blood following consumption of large amounts of protein. Vitamin B₆ also can increase the metabolism of levodopa by enhancing its conversion to dopamine before the drug crosses the blood-brain barrier. Vitamin B₆ is found in liver and other protein-rich foods, such as meats and legumes, as well as seeds and whole grains. Thus, ingesting levodopa with large amounts of protein or vitamin B₆, or regularly consuming a diet high in protein or vitamin B₆ while taking levodopa, is contraindicated.

Effects of Foods/Nutrients on the Actions of Drugs

Some foods or nutrients can enhance or oppose the actions of drugs. Foods that contain amines, especially tyramine, dopamine, or histamine, are known to interact with a group of drugs known as monoamine oxidase inhibitors (MAOIs), which are used mostly to treat some forms of depression [5]. MAOIs such as Parnate (tranylcypromine sulfate) and Nardil (phenelzine sulfate) prevent the enzyme monoamine oxidase from catabolizing amines in the diet as well as amines made endogenously. Amines consist of vasoactive or pressor amines (e.g., tyramine, serotonin, and histamine) and neurotransmitters or psychoactive amines (e.g., dopamine and norepinephrine). The antituberculosis drug INH (isoniazid) exhibits MAOI-like activity. The problem arises when people on MAOIs or INH eat foods high in amines, especially tyramine or histamine, which may be found in fairly large quantities in some foods. Consuming these foods ordinarily presents no problem, because the amines can quickly be inactivated by monoamine oxidase (MAO). However, in people taking MAOIs or INH, these reactions do not occur. Consequently, high dietary amine intake coupled with

high endogenous norepinephrine may result in excessive vasoconstriction, manifested as severe headache, acute hypertension or a hypertensive crisis, and cardiac dysrhythmia. People taking MAOIs are counseled against ingesting foods high in amines, such as aged cheeses (cheddar, Camembert, Stilton, Boursault), yeast extracts (e.g., Marmite), and brewer's yeast. Smoked, salted, or pickled fish such as herring or cod, as well as sausage, salami, pepperoni, corned beef, and bologna, also are high in tyramine. Foods moderately high to high in tyramine include meat extracts; tenderizers; red wines, including Chianti, vermouth, sherry, and burgundy; and cheeses such as blue, natural brick, Brie, Gruyère, mozzarella, Parmesan, Romano, and Roquefort. Broad beans (fava, Chinese pea pods), chocolate, large amounts of caffeine, liver (chicken or beef), and selected fruits may also contain large amounts of tyramine. Histamine is not typically found in large quantities in foods, with the exception of improperly stored or spoiled fish. Dopamine is found in fava and broad beans and snow peas.

A nutrient known to antagonize the action of the anticoagulant Coumadin (warfarin) is vitamin K [6]. Coumadin works by inhibiting reactions in the vitamin K cycle that generate the active form of vitamin K needed for blood clotting (Figure 10.24). By inhibiting production of active vitamin K, the drug prolongs the clotting time of blood. Large amounts of vitamin K oppose the actions of the drug, promoting blood clotting and leading to drug resistance. Ingesting large quantities of foods rich in vitamin K, including green vegetables, some legumes (soybeans, garbanzo beans), and liver should be avoided.

Caffeine, a component of coffee, tea, many soft drinks, and chocolate, counters the actions of tranquilizers and may exacerbate some adverse effects of the bronchodilators: Theo-24, Theolair, Uniphyl, and Elixophyllin (theophylline). Specifically, large amounts of caffeine coupled with use of theophylline promote increased nervousness, insomnia, and tremors. (These drugs also interfere with vitamin B₆ metabolism.)

Effects of Foods/Nutrients on Drug Excretion

Sodium and the mineral lithium in the antimanic drugs Eskalith, Lithobid, or Lithotabs (lithium carbonate) are known to interact in the kidneys. Specifically, the sodium and lithium compete with each other for reabsorption into the tubules of the kidneys. Thus, high intake of sodium promotes lithium excretion and thereby diminishes the effects of the drug, whereas low intake

of sodium promotes reabsorption of lithium and thereby enhances the likelihood of drug toxicity.

Effects of Drugs on Nutrient Absorption

Drugs may alter the absorption of nutrients through several mechanisms. For example, drugs may alter the transit time of nutrients through the gastrointestinal tract, speeding up or slowing down the passage of its contents. Typically, when contents move quickly through the gastrointestinal tract, fewer nutrients are absorbed. Alternately, when contents move slowly through the gastrointestinal tract, more nutrients are absorbed. Changes in the pH of the gastrointestinal tract also may alter nutrient absorption. For example, H₂ receptor blockers such as Tagamet (cimetidine) and Zantac (ranitidine) and proton-pump inhibitors such as Prilosec (omeprazole) and Prevacid (lansoprazole), which decrease hydrochloric acid secretion into the stomach and thus increase gastric pH, diminish the absorption of several nutrients, especially vitamin B₁₂ and iron. H₂ receptor blockers and proton pump inhibitors are used to treat ulcers and gastroesophageal reflux disease (GERD).

The ability of some drugs to chelate or adsorb nutrients or gastrointestinal secretions also diminishes nutrient absorption. For example, bile acid sequestrants such as Questran (cholestyramine) adsorb bile and thus decrease the absorption of carotenoids and the fat-soluble vitamins A, D, E, and K. In addition, the drug chelates other nutrients (including folate) and some divalent minerals (including iron, magnesium, calcium, and zinc). Bile acid sequestrant drugs are used to treat high blood cholesterol concentrations, a risk factor for heart disease.

Effects of Drugs on Nutrient Metabolism

In addition to altering nutrient absorption, drugs may alter the metabolism of nutrients in body tissues. INH (isoniazid), used in the treatment of tuberculosis, for example, diminishes the conversion of pyridoxine (vitamin B₆) to its functional coenzyme form in the liver and thus can cause a vitamin B₆ deficiency. Another group of drugs known to alter vitamin metabolism includes the anticonvulsants phenobarbital and

metabolism of vitamin D, leading to (in severe cases) the deficiency conditions rickets and osteomalacia if vitamin supplements (as 25-OH cholecalciferol) are not given. Specifically, the anticonvulsants are thought to diminish the hepatic conversion of vitamin D as

cholecalciferol to 25-OH cholecalciferol. Interestingly, these anticonvulsants also affect the vitamin folate by diminishing its absorption in the intestine.

Effects of Drugs on Nutrient Excretion

Drugs may increase or decrease the excretion of nutrients from the body. Diuretics such as Lasix (furosemide), Bumex (bumetanide), and other loop diuretics used to treat high blood pressure promote the urinary excretion of sodium and water (important in lowering blood pressure); however, the drugs also increase losses of potassium, chloride, and magnesium from the ascending portion of the loop of Henle. Dietary replacement of the minerals, especially potassium, is important to prevent low blood potassium concentrations.

Summary

Nutrient-drug interactions can severely affect both nutritional status and the effectiveness of pharmacological treatment. Although accredited health-care facilities are mandated to educate patients about interactions between foods and drugs, many individuals remain unaware of such interactions and their consequences.

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13

Ultratrace Elements

Arsenic

Boron

Nickel

Silicon

Vanadium

Cobalt

For each of the ultratrace elements listed, the following subtopics (if known and when applicable) are discussed:

Sources

Absorption, Transport, Metabolism, and Storage

Functions and Deficiency

Interactions with Other Nutrients

Excretion

Recommended Intake, Toxicity, and Assessment of Nutriture

Ultratrace elements have been defined as those elements with estimated, established, or suspected requirements of <1 mg/day [1]. Based on this definition, as many as 19 elements may be classified as ultratrace elements: aluminum, arsenic, boron, bromine, cadmium, chromium, copper, fluoride, germanium, iodine, lead, lithium, molybdenum, nickel, rubidium, selenium, silicon, tin, and vanadium [2]. However, while copper, chromium, fluoride, iodine, molybdenum, and selenium are considered an ultratrace element by the above definition, they have been included in Chapter 12, “Microminerals,” based on the establishment of either an AI or an RDA by the Food and Nutrition Board [3].

The ultratrace elements—arsenic, boron, nickel, silicon, and vanadium—which are addressed in this chapter are not, at present, considered essential, although for each, some evidence suggests a possible need [1–3]. As in Chapters 9–12, this chapter covers, for each of the five ultratrace elements, its sources, absorption, transport, mechanisms, storage, functions, interactions with other nutrients, excretion, recommended intake, deficiency, toxicity, and how nutriture is assessed. Table 13.1 provides an overview of selected functions, food sources, and deficiency symptoms for these nutrients. Figure 13.1 shows the location of these elements in the periodic table. A brief discussion also is provided for the element cobalt, which is needed in the body only as part of vitamin B₁₂ (cobalamin).

Table 13.1 Ultratrace Elements: Selected Functions, Deficiency Symptoms, and Food Sources

Mineral	Selected Possible Physiological Roles	Selected Deficiency Symptoms in Animals	Food Sources
Arsenic	Methyl group use, normal growth	Curtailed growth	Seafood
Boron	Bone development, cell membrane, embryogenesis, metabolic regulator, inflammation	Altered bone mineral metabolism, depressed growth	Fruits, vegetables, legumes, nuts
Nickel	Possibly involved in hormonal membrane or enzyme activity	Depressed growth, impaired hematopoiesis	Nuts, legumes, grains, cacao products
Silicon	Connective tissue and bone formation, prolylhydroxylase activity	Decreased collagen, long bone and skull abnormalities	Beer, unrefined grains, root vegetables
Vanadium	Mimics insulin action, inhibition of Na ⁺ /K ⁺ -ATPase	Reduced growth, hematologic changes, metabolism changes	Shellfish, spinach, parsley, mushrooms, whole grains

Figure 13.1 The periodic table highlighting important ultratrace elements.

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Arsenic

More than any other ultratrace mineral, arsenic, which is colorless and odorless, conjures an image of toxicity as a poison rather than of nutritional essentiality. The malevolent aspect of arsenic continues to attract attention because much more of the arsenic literature addresses its toxicological rather than its nutritional properties. Nevertheless, evidence is accumulating that arsenic may be an essential element.

SOURCES

Arsenic is present throughout the earth's continental crust at an estimated concentration of 1.5–2.0 µg/g. It is present

in water, rocks, and soils, although its concentration varies considerably among regions, based on the geological history of the soil as well as on pollution from unnatural sources. Fallout sources such as pesticides, smelters, and coal-fired power plants can, through aerosols and floating dust, enrich a particular area with arsenic, which then affects humans and animals when it is incorporated into water, foods, and foodstuffs. Foods of marine origin are rich in arsenic, with fish containing up to 80 µg/g and oysters up to 10 µg/g [1,2]. Arsenic is also present in meats (0.005–0.1 µg/g) and cereal and grain products (0.05–0.4 µg/g) as well as dairy (milk, 0.01–0.05 µg/g; eggs, 0.01–0.1 µg/g) [2]. Dietary intakes of arsenic usually total <30–50 µg/day [1,2].

Arsenic is found in water and foods in organic and inorganic forms, and exists mostly in trivalent arsenic [As^{3+}] and pentavalent arsenic [As^{5+}]. The major arsenicals found in water and foods are pentavalent arsenate (H_2AsO_4^- or HAsO_4^{2-}) and trivalent arsenite (H_3AsO_3 and H_2AsO_3^-). Foods also contain arsenic in methylated forms. The arsenicals in foods include monomethylarsonic acid, dimethylarsinic acid, trimethylarsine [$\text{As}(\text{CH}_3)_3$], arsobetaine, arsenocholine, trimethylarsonium lactate, and O-phosphatidyltrimethylarsonium lactate (Figure 13.2). Of the arsenicals, inorganic arsenite and trivalent organo-arsenicals are the most toxic, while the pentavalent, methylated arsenic compounds are less toxic.

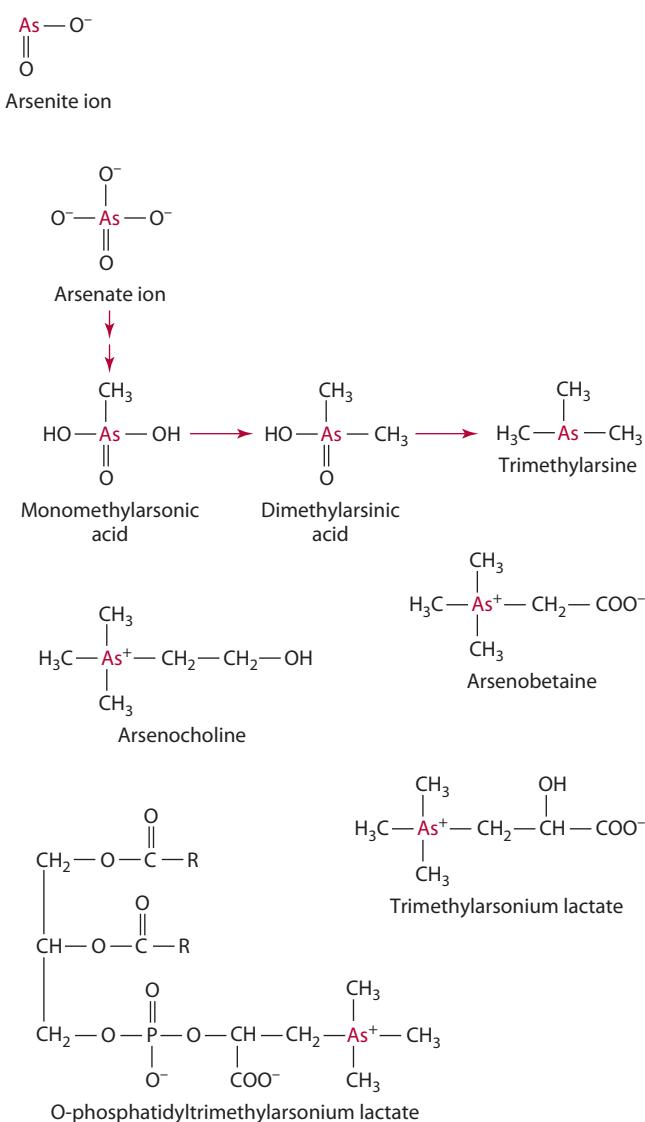


Figure 13.2 Forms of arsenic of biological importance.

ABSORPTION, TRANSPORT, AND METABOLISM

Absorption, as well as retention and excretion, of arsenicals varies with their chemical form and solubility, the quantity ingested, and the animal species. Most inorganic arsenic is absorbed as arsenate and arsenite; arsenate is the less toxic form [3]. Greater than 90% of inorganic arsenate and arsenite is absorbed from water, and between 60% and 75% of these inorganic forms of the element is absorbed with food in humans [3]. Similarly, of the organic arsenicals, >90% of arsenobetaine and between 70% and 80% of arsinocholine are absorbed.

Absorption of organic and inorganic arsenicals is thought to occur by simple diffusion across the intestinal mucosa. Typically, the greater the lipid solubility of the arsenical, the greater the likelihood that it is absorbed by simple diffusion. From the intestine, arsenic

is transported in the blood to the liver, which takes up both inorganic and organic forms of the element following absorption. In the liver, organic arsenic, such as arsenobetaine or arsinocholine, is thought to undergo little or no metabolism. In contrast, inorganic arsenic is extensively reduced, methylated, or both. In the liver, arsenate [As^{5+}] is reduced using glutathione or other thiols to the more toxic trivalent arsenite, which is then methylated (using S-adenosylmethionine, SAM) to become monomethylarsonic acid. The reaction is catalyzed by arsenite methyltransferase. Inorganic arsenic also can be methylated to monomethylarsonic acid. In addition, another methyl group may be added to monomethylarsonic acid by monomethylarsonic acid methyltransferase to form dimethylarsinic acid. Methyl groups are provided by glutathione, choline, and S-adenosylmethionine [4]. Dimethylarsinic acid can be reduced to form a relatively toxic compound dimethylarsenous acid; this event typically represents the last step in arsenic metabolism, but some arsenic may remain as inorganic arsenic and as monomethylarsonic, with metabolism depending in part on folate's role in one carbon metabolism [5]. The general order of toxicity of arsenicals is monomethylarsonic acid⁽³⁺⁾ > inorganic As^{3+} > inorganic As^{5+} > monomethylarsonic acid⁽⁵⁺⁾ = dimethylarsinic acid⁽⁵⁺⁾ [6]. Thomas and coworkers provide a review of arsenic methylation and toxicity for the interested reader [7]. In systemic blood, arsenic is found in two forms, methylated and protein-bound.

Tissues that contain the most arsenic include skin, hair, and nails. Within tissues, inorganic arsenic, especially as As^{3+} , is found bound primarily to thiol/sulfhydryl (SH) groups of proteins, such as the zinc storage protein metallothionein [8,9]. Methylated forms of the element do not bind to tissues as much as unmethylated inorganic forms.

FUNCTIONS AND DEFICIENCY

Arsenic appears to be needed to form and use methyl groups, generated in methionine metabolism to S-adenosylmethionine (SAM). SAM is a major methyl donor in the body and functions in synthesizing a variety of compounds and in methylating compounds needed for DNA synthesis. Arsenic has not, however, been shown to activate or inhibit a specific enzyme in methionine metabolism.

Arsenic deficiency impairs metabolism of methionine, resulting in decreased SAM concentrations and decreased S-adenosylmethionine decarboxylase activity [10]. Similarly, taurine production from methionine is depressed in arsenic-deficient rats and hamsters [11]. Arsenic-deficient rats fed guanidoacetate (needed for creatine synthesis) experienced growth deficits compared with arsenic-supplemented rats [12]. Decreased methylation of guanidoacetate to form creatine and decreased methylation of histones and DNA also are reported with arsenic deprivation [12,13]. Synthesis of

polyamines, which for example are derived from SAM, is impaired with arsenic deficiency. Synthesis of heat shock proteins, through effects on methylation of histones, has been attributed to arsenic's roles in gene expression [13,14]. Other reported effects of arsenic deprivation in animals include curtailed growth, reduced conception rate, and increased neonatal mortality.

INTERACTIONS WITH OTHER NUTRIENTS

Arsenic seems to interact antagonistically with selenium and iodine. Because selenate and arsenate are both oxyanions with similar chemical properties, each may competitively inhibit the uptake and tissue retention of the other. The interaction of arsenic with iodine is exemplified by the observation that it is goitrogenic in mice. Arsenic is believed to antagonize the mechanism of iodine uptake by the thyroid, causing compensatory goiter.

EXCRETION

Ingested arsenic is excreted rapidly by the kidneys, which represent the major route of excretion. The main urinary metabolites of arsenic include monomethylarsinic acid, dimethylarsinic acid, and trimethylated arsenic [15,16]. When organic forms of the element are consumed, arsobetaine and arsenocholine, along with arenosugars, are excreted in the urine [8,17]. Concentrations of the metabolites in the urine, however, vary depending on the dietary form of arsenic ingested [15]. Typically, <50 µg arsenic is excreted in the urine of healthy adults each day.

RECOMMENDED INTAKE, TOXICITY, AND ASSESSMENT OF NUTRITION

Insufficient data are available to estimate a human dietary requirement for arsenic, although a requirement of 12–25 µg has been suggested [11,12,18]. No tolerable upper intake level for arsenic has been established by the Food and Nutrition Board [19].

Inorganic forms of arsenic are more toxic than organic forms of the element and appear to be carcinogenic; susceptibility to toxicity, however, appears to relate in part to nutritional status [5,20,21]. Acute toxicity results in gastrointestinal distress (leading to dehydration and electrolyte imbalance), encephalopathy, anemia, and hepatotoxicity. Arsenic is fatal at intakes of 70–300 mg [22]. Chronic toxicity is associated with skin hyperpigmentation, hyperkeratosis, muscle weakness, peripheral neuropathy, excessive sweating, liver damage, delirium, encephalopathy, vascular changes, and cancers of the oral cavity, skin, lungs, colon, bladder, and kidney [23–25]. The toxicity relates in part to arsenic's interactions with sulfhydryl groups found in proteins (including enzymes), which results in the formation of free radicals that damage cells. In Taiwan, ingesting

arsenic-containing drinking water has been associated with blackfoot disease, a peripheral vascular condition [23,24]. See the article by Duker and others for a review of arsenic toxicity [26].

Reported levels of arsenic in body fluids range from 2–62 ng/mL in whole blood and 1–20 ng/mL in plasma or serum. Arsenic levels in hair range from ~0.1–1.1 µg/g. Chronic or acute exposure to the metal elevates these values. Hair analysis has been particularly useful in this respect, because hair arsenic content, unlike that of the fluids, represents an average content over an extended period and does not fluctuate if exposure to the element is intermittent.

The current method of choice for determining arsenic in biological fluids is atomic absorption spectrometry, although mass spectrometry, neutron activation analysis, and emission spectroscopy have been used successfully.

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Boron

Boron, as boric acid and sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot \text{H}_2\text{O}$, called borax), was used to preserve foods such as fish, meat, cream, butter, and margarine for over 50 years—that is, until about the 1920s, when it was considered dangerous for humans but deemed essential for plants. Not until the 1980s did evidence for the essentiality of boron in animals start mounting again.

SOURCES

Foods of plant origin such as fruits, vegetables, nuts, and legumes are particularly rich in boron [1–3]. In addition, wine, cider, and beer contribute to dietary intake. Specific foods particularly rich in boron include avocado, peanuts, peanut butter, pecans, raisins, grapes, and wine [4,5]. Generally, raisins, legumes, nuts, and avocados provide ~1.0–4.5 mg boron/100 g, and fruits and vegetables contain 0.1–0.6 mg boron/100 g [4,6,7]. Meat, fish, and dairy products are poor sources of the element, usually providing <~0.6 mg boron/100 g [1–3,7]. Drinking water and water-based beverages vary considerably in boron content based on geographic location [3]. Boron also is a contaminant or a major ingredient in some antibiotics, gastric antacids, lipsticks, lotions, creams, and soaps, for example [3]. Boron appears in foods as sodium borate or as organic borate esters [5,8]. Dietary intake of boron is estimated at 0.8–1.5 mg/day [4,8].

ABSORPTION, TRANSPORT, STORAGE, AND EXCRETION

Greater than 85% of ingested boron is thought to be absorbed as boric acid $\text{B}(\text{OH})_2$ and orthoboric acid $\text{B}(\text{OH})_3$

by passive diffusion from the gastrointestinal tract [8,9]. Boron is found in the blood as boric acid, orthoboric acid, and the borate monovalent anion $\text{B}(\text{OH})_4^-$. A borate transporter appears to actively transport $\text{B}(\text{OH})_4^-$ into cells against a concentration gradient. Boron is found mainly in bone, teeth, nails, and hair. Total body boron content ranges from ~3–20 mg. It is excreted primarily (>70%) in the urine, with <13% usually lost in the feces and small amounts lost in sweat [8]. Urinary boron appears as boric acid and orthoboric acid, and is thought to be a relatively sensitive indicator of intake within an intake range of 0.35–10 mg boron/day [8].

FUNCTIONS AND DEFICIENCY

Boron is thought to have several functions in the body, including roles in embryogenesis, bone development, cell membrane function and stability, metabolic regulation, and the immune response. For example, with respect to embryogenesis, in some but not all animals (e.g., not in rodents) boron depletion has resulted in embryonic and developmental defects, suggesting a possible role in reproduction or development. Depressed growth is also frequently observed in boron-depleted animals [2,10]. The composition, structure, and strength of bones also appear to involve boron, possibly through modulation of extracellular matrix turnover. For example, boron enhanced collagenase and cathepsin D activity in fibroblasts to modulate the turnover of the extracellular matrix [11]. Boron also enhanced the actions of estradiol on trabecular bone and promoted absorption and retention of minerals in bone in ovariectomized rats [12]. With respect to bone mineral content, responses to boron have been most marked in animals that also have had calcium, vitamin D, or magnesium deprivation [13].

In addition to effects on embryogenesis and bone, boron may play a role in cell membrane stability, function, or both [14,15]. Boron may affect the cell membrane by modulating calcium uptake into the cell or by modulating the ability of hormones (including vitamin D, calcitonin, insulin, and estrogen) to bind to receptors and to exert their actions [10,14]. Boron also may affect cell membrane function or stability through effects on transmembrane signaling [10,14,15].

As a metabolic regulator, boron decreases serum glucose and increases serum triglyceride concentrations. Boron may directly interact with nutrient substrates, enzymes, or coenzymes or exert its effects through other mechanisms [16,17]. Boron-deficient chicks, for example, exhibit increased insulin secretion, possibly mediated through effects of boron on ion transport [14,17].

Finally, boron is thought to regulate the body's inflammatory response. As a mediator of the inflammatory process, boron may suppress (through reversible formation of analogues and competition with coenzymes) the activities of

several serine proteases (e.g., elastase, chymase, and cathepsin) released by inflammation-activated white blood cells [17]. Alternately, boron may decrease leukotriene synthesis or decrease the generation or removal of reactive oxygen species produced by neutrophils as part of the respiratory burst [17]. Other studies suggest that boron acts by suppressing T-cell activity and altering antibody concentrations [17]. In addition to acting on serine proteases involved with the inflammatory response, boron is thought to affect other serine proteases, such as those involved in blood coagulation [17]. Because of its anti-inflammatory effects, boron is purported to reduce the severity of rheumatoid arthritis (among other inflammatory conditions).

RECOMMENDED INTAKE, TOXICITY, AND ASSESSMENT OF NUTRITION

A recommended intake of boron has not been established. Dietary intakes of <1 mg have been reported, but the amount needed to meet requirements is unknown [4,8].

Acute boron toxicity results in nausea, vomiting, diarrhea, dermatitis, and lethargy [18]. Increased urinary excretion of riboflavin also has been reported with boron toxicity [19]. Chronic boron toxicity is associated with nausea; poor appetite and subsequent weight loss; anemia; patchy, dry erythema; and seizures [20]. A tolerable upper intake level of 20 mg boron per day has been established for adults, based on animal studies [21].

Inductively coupled plasma emission spectrometry has been used to determine plasma and other body fluid concentrations of boron; however, whether these tissue concentrations indicate nutritional status is unknown. Plasma boron concentrations usually range from ~20–75 ng/mL [22].

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Nickel

Nickel is used industrially in various capacities, such as production of stainless steel and nickel-cadmium batteries. Nickel is released into the environment when nickel-containing products are burned. Nickel's essentiality in human nutrition was first suggested in the 1930s; however, not until the mid-1970s did studies focus on its possible roles.

SOURCES

Foods of plant origin have a substantially higher nickel content than foods of animal origin. Nuts, legumes, grains and grain products, and chocolate (and items made from chocolate) are particularly rich in the metal, providing up to ~228 µg/100 g [1]. Fruits and vegetables generally have intermediate nickel content, providing up to ~48 µg/100 g [1]. Nickel content of foods of animal origin, such as fish, milk, and eggs, generally is low [1,2]. Total daily dietary intake of nickel by adults is typically <100 µg [1,2].

The chemical form of nickel in foods is unknown, but in plants it is probably largely inorganic and depends on the nickel content of the soil. Dietary nickel derived from contamination of processed foods is likely inorganic as well.

ABSORPTION, TRANSPORT, AND STORAGE

Nickel absorption from foods is thought to be <10%. Absorption of nickel is higher (about doubled, but it can be up to 50%) from water than from other beverages (such as coffee, tea, cow's milk, and orange juice) to which nickel has been added [2–4]. With ingestion of high doses of nickel (up to 50 µg/kg body weight), nickel absorption was $27 \pm 17\%$ following an overnight fast and $0.7 \pm 0.4\%$ with a meal [3].

Nickel is absorbed across the intestinal brush border by a carrier and passive diffusion [5,6]. In fact, nickel ions compete with iron for carrier transport in the proximal small intestine [6,7]. Thus, absorption of nickel increases with iron deficiency. Transport across the basolateral membrane is thought to occur by diffusion or as part of a complex with an amino acid or binding ligand.

In the blood, nickel binds mainly to albumin and to a lesser extent to amino acids, including histidine, cysteine, and aspartic acid [8]. Other serum proteins, such as α -2 macroglobulin, also may transport nickel in the blood. Uptake of nickel into cells may occur with amino acids, with transferrin, or through a divalent cation channel such as Ca^{2+} .

Although nickel is widely distributed among human tissues, its concentration throughout the body is extremely low, occurring at nanogram/gram levels. The highest concentrations of nickel are found in the thyroid and adrenal glands as well as in hair, bone, and soft tissues such as the lungs, heart, kidneys, and liver.

FUNCTIONS AND DEFICIENCY

A specific role of nickel in human and animal nutrition has not yet been defined, although roles for nickel in plants and microorganisms have been documented [9]. In plants, for example, nickel serves as a cofactor for urease, which catalyzes the hydrolysis of urea into carbon dioxide and ammonia. In bacteria, several hydrogenases appear to be nickel dependent. In the several enzyme systems, however, the role of nickel can be substituted for other minerals, such as magnesium. An example of such a replacement is the formation of the C3 convertase enzyme (C3b,Bb and C4b,2b) of the human complement system, which classically requires Mg^{2+} for activity. The substitution of nickel for magnesium in this complex enhanced both the stability and the activity of the enzyme, raising a question as to nickel's possible physiological role in the complement system [10]. It has also been demonstrated that nickel can substitute for zinc in the carboxypeptidases and in horse

liver alcohol dehydrogenase [9]. Nickel may be involved with folate and vitamin B_{12} in the metabolism of methionine, either in the initial stages involving conversion to homocysteine, or with vitamin B_{12} in the later stages, during which propionyl CoA is converted to succinyl CoA [11,12].

Signs of nickel deprivation continue to be described for some animal species. Among the more consistent signs are depressed growth, altered distribution of some minerals, changes in blood glucose, and impaired hematopoiesis, which probably is caused by altered iron metabolism.

INTERACTIONS WITH OTHER NUTRIENTS

Because nickel shares with other metals the property of being readily chelated by and complexed with a wide variety of ligands, it follows that nickel can compete with those metal ions for ligand sites. Nickel interacts with many ions in this manner, including as many as 13 essential minerals. The interactions of particular nutritional interest are those involving iron, copper, and zinc. As described in the section on absorption, nickel antagonizes ferrous iron absorption [6,7]. Nickel is thought to replace copper at functional sites and thus exacerbates copper deficiency [13]. With respect to zinc, nickel appears to affect zinc metabolism, possibly by causing redistribution of zinc in the body [13].

EXCRETION

Most absorbed nickel is excreted in the urine in amounts less than about 10 µg/L [14]. Within the renal cells, nickel is first complexed with low molecular-weight compounds such as uronic acid and neutral sugar oligosaccharides. Small amounts (1.5–3.3 µg/day) of absorbed nickel also are excreted through the bile [15]. Sweat nickel concentrations, however, can be fairly high (up to 69.9 µg/L) with active secretion of the element by sweat glands, even in acclimatized individuals [16].

RECOMMENDED INTAKE, TOXICITY, AND ASSESSMENT OF NUTRITION

Extrapolation from animal studies suggests that humans probably need <100 µg of nickel per day [14]. A tolerable upper intake level for adults for nickel is 1.0 mg/day in the form of soluble nickel salts (such as nickel sulfate, which may contaminate water) [17].

Signs of toxicity in humans include nausea, vomiting, and shortness of breath; in animals, signs include lethargy, ataxia, irregular breathing, and hypothermia, among others, possibly including death [17]. Nickel is also a known carcinogen with demonstrated effects on DNA, including hypermethylation of DNA, inhibition of acetylation of histones, condensation of chromatin, and gene silencing [18].

As for most of the ultratrace metals, the preferred technique for nickel determination is flameless atomic absorption spectrophotometry. This technique offers the degree of sensitivity necessary for determination in the nanogram range. The reference range for nickel in the serum or plasma of healthy adults is 1–23 ng/mL; however, valid methods to assess the nickel status of humans are unavailable.

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Silicon

Silicon occupies a unique position among the essential trace elements in that it is second only to oxygen in earth-wide abundance. Quartz, which is crystallized silica, is the most abundant mineral in the earth's crust. The element

occurs naturally as silicon dioxide or silica, SiO_2 , and as water-soluble ortho- or monosilicic acid, $\text{Si}(\text{OH})_4$, formed by hydration of the oxide. In plants, silicon is deposited as the solid, hydrated oxide $\text{SiO}_2 \cdot n\text{H}_2\text{O}$, known as silica gel, following polymerization of silicic acid. Some other forms of silica include talc, clay, asbestos, and glass.

Whereas early investigations concentrated on silicon's toxicity, such as silicon-related urolithiasis (stones in the urinary tract) and particularly silicosis (a respiratory condition caused by the inhalation of dust), since about the mid-1970s research has focused on the possible roles or functions of silicon in animals and humans.

SOURCES

Data on the distribution of silicon in human foods and diets are sparse. It is known, however, that foods of plant origin normally are much richer in silicon than those of animal origin. Whole cereal grains and root vegetables appear to be especially rich sources of the element, providing about 14% and 8%, respectively, of intake [1,2]. Silica is also in water, and thus beverages, such as water and coffee; it is also in beer, because of the hops and barley [1]. Silicon intakes by adults are estimated to range from about 14–62 mg/day [1,3,4].

ABSORPTION, TRANSPORT, STORAGE, AND EXCRETION

The mechanism of silicon absorption is not well understood, and future studies will likely be complicated by its diverse dietary forms. Silica, monosilicic acid, phytolithic silica, and silicon found in organic combination (e.g., with pectin and mucopolysaccharides) are a few of its ingestible forms. In fluids, silicon is found as orthosilicic acid, $\text{Si}(\text{OH})_4$. The most soluble form of silicon is metasilicate, which as sodium metasilicate has commonly been used in supplementation studies.

Overall estimates of the absorption of silicon range from 1% to >70% depending on the form of silicon ingested, solubility in the gastrointestinal tract, and the presence of fiber [5,6]. For example, nearly 97% of dietary silicon contained in a high-fiber diet remained unabsorbed and was lost in the feces, compared to a fecal excretion of only 60% when a low-fiber diet was consumed [2]. Silicon absorption from fluids is estimated at >50%. In studies in animals, absorption also appears to be affected by age, sex, and various hormones [7]. In addition, molybdenum intake inhibits silicon absorption, and vice versa.

Silicon in the body is found bound as well as in free forms such as orthosilicic acid, $\text{Si}(\text{OH})_4$ [8]. Once silicic acid is absorbed into the blood, it is almost entirely free (i.e., not bound to proteins), thus accounting for its rapid decrease in plasma concentration, its diffusion into tissue fluids, and its rapid urinary excretion [9]. After intravenous administration of ^{31}Si silicic acid, the label was most rapidly taken

up by liver, lung, skin, and bone, with slower entry occurring in heart, muscle, spleen, and testes. Negligible uptake into the brain was reported, indicating active exclusion by the blood-brain barrier. Generally, silicon concentrates in the body's connective tissues, such as bone, skin, blood vessels (e.g., the aorta), and tendons.

The kidney is the major excretory organ of absorbed silicon; 77% of labeled silicic acid was excreted in the urine of rats within 4 hours [9]. Urinary silicon excretion is significantly correlated with silicon intake [4]. Most silicon is thought to be excreted in the urine as orthosilicic acid and as magnesium orthosilicate.

FUNCTIONS AND DEFICIENCY

The physiological role of silicon centers on normal formation, growth, and development of bone, connective tissue, and cartilage. Silicon is thought to play both a metabolic and a structural role. In bone, silicon influences bone formation and growth processes, including bone mineralization and crystallization [10–14]. Silicon deficiency results in smaller, less flexible long bones and in skull deformation. In studies on chicks, the skull deformation was subsequently found to be caused by reduced collagen in the connective tissue matrix [10]. Decreases in femoral and vertebral calcium, copper, potassium, and zinc concentrations and increased plasma alkaline phosphatase activity have been reported with silicon deprivation in rats [12]. Moreover, silicon deprivation in rats diminished bone collagen formation (with decreased activity of ornithine aminotransferase, an enzyme required for collagen formation) and increased collagen breakdown [13]. In culture cells, orthosilicic acid stimulated collagen synthesis and bone cell differentiation [14].

RECOMMENDED INTAKE, TOXICITY, AND ASSESSMENT OF NUTRITION

The minimum silicon requirement compatible with human health is largely unknown, although estimates of a human requirement based on animal studies range from 2–5 mg/day and recommendations for intake for humans range from ~5–35 mg/day [4,5,15]. Using urinary silicon excretion as a basis for estimates of requirements for humans, Carlisle suggested a requirement between 10–25 mg/day [15].

No tolerable upper intake level has been established for silicon, although a safe upper level of 1,750 mg per day has been suggested [16]. The major potential adverse effect reported is kidney stones; however, it is frequent, chronic (years) use of large amounts of silicon-containing antacids (e.g., magnesium trisilicate, which can provide 6.5 mg elemental silicon per tablet) that appears to be related to a few cases of kidney stones [3,16,17]. Toxicity of silicon also has been associated with diminished activities of several enzymes that prevent free radical damage, including

glutathione peroxidase, superoxide dismutase, and catalase [18]. Silicosis occurs from inhaling dust high in silica; the condition is characterized by a progressive fibrosis of the lungs that leads to respiratory problems.

As in the case of most of the trace elements, levels of silicon in biological fluids of healthy adults have been reported but may not accurately represent nutriture. Chemical assessment generally is performed on serum or plasma, which contains about 50 µg silicon/dL [16]. Mass spectrometry, emission spectroscopy, and atomic absorption spectrophotometry are a few of the techniques for determining silicon concentration in biological specimens. Of these, atomic absorption spectrophotometry has been the method of choice for most laboratories.

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Vanadium

Vanadium was first discovered in the early 1800s and named for a Swedish goddess, Vanadis [1]. The element exists in several oxidation states from V^{2+} to V^{5+} . In solution, vanadium produces a range of colors, which accounts for its being named after the goddess. In its pentavalent state it is yellowish orange, whereas in its divalent state it is blue [1]. V^{3+} has been shown to form complexes with amino acids such as alanine and aspartate [2]. In biological systems including the serum, vanadium is found primarily in the pentavalent state, V^{5+} , known as vanadate or monovanadate (VO_3^- , VO_4^{3-} , or $H_2VO_4^-$), or in the tetravalent state, V^{4+} , known as vanadyl ion (VO^{2+}). In acidic conditions, it is found as vanadyl VO^{2+} and in alkaline conditions as the ortho monovanadate ion VO_4^{3-} .

SOURCES

The content of vanadium in foods is very low, and consequently so is the average dietary intake. Most fats and oils contain particularly low levels of the mineral, <0.3 µg/100 g [3]. A few items, including black pepper, parsley, dill seed, canned apple juice, fish sticks, and mushrooms, contain relatively high concentrations, and shellfish such as oysters are particularly rich in the element, with up to ~12 µg/100 g [3,4]. Cereals and grain products contribute fairly substantial amounts of vanadium (up to 15 µg/100 g) to the diet, as do sweeteners (up to 4.7 µg/100 g) [4]. Beer and wine also provide a source of vanadium [3,4]. Vanadium intake in the U.S. diet is thought to range from 10–60 µg/day, with many diets containing <~15 µg [1,3,4]. Supplements providing vanadium as vanadyl sulfate and sodium metavanadate are available.

ABSORPTION, TRANSPORT, AND STORAGE

Absorption of vanadium varies with its oxidation states. For example, vanadate is thought to be reduced to the vanadyl ion (VO^{2+}) in the stomach before being absorbed in the upper small intestine. Compared to vanadyl, the vanadate anion is three to five times more efficiently absorbed, likely because it is transported by the transport system for phosphate, which it mimics chemically [5]. Overall, vanadium absorption is generally <5% [5]. However, studies on vanadium absorption in rats have documented absorption ranging from 10% to as high as 40% [6]. These rat studies suggest caution in assuming that vanadium is always poorly absorbed.

In blood cells and in plasma and other body fluids, vanadate is converted to vanadyl. Glutathione, NADH, and ascorbic acid can act as reducing agents for vanadate. In the plasma, vanadyl binds to albumin and iron-containing proteins such as transferrin and ferritin [7]. Studies show that V^{3+} exhibits greater binding to the

N-terminal end of transferrin than V^{4+} and V^{5+} ; however, whether significant quantities of V^{3+} are present in biological systems is unclear [8].

Vanadium is thought to enter cells as vanadate (HVO_4^{2-}) through transport systems for phosphate and possibly other anions, or as vanadate VO_3^- through an ion transport channel. Similar to reactions in the plasma, intracellular vanadate is reduced primarily by glutathione to vanadyl, which is then almost exclusively bound to a variety of ligands, many of which are phosphates and iron-containing proteins; <1% remains unbound [9]. Vanadyl may be converted back to vanadate by NADPH oxidation pathways [10].

Little vanadium is found in the body; the total body pool of vanadium is about 100–200 µg [3,7]. Most tissues contain <10 ng V/g tissue [11,12]. Distribution studies indicate that although kidney cells retain most of the absorbed mineral soon after it is administered, accumulation later shifts principally to bone and teeth, lungs, and thyroid gland, with somewhat lesser amounts in the spleen and liver. This shift is understandable in view of the high content in bone of inorganic phosphate, to which vanadyl binds tenaciously [9].

FUNCTIONS

Vanadium is very active pharmacologically, exerting a broad assortment of effects that are well documented. However, take care not to confuse essentiality with pharmacological activity, because pharmacological activity is generally manifested only above a concentration threshold that is considerably greater than that required to fulfill the need for essentiality.

No specific biochemical function has been identified for vanadium. Many of vanadium's effects *in vivo* are predictable from a consideration of its aqueous chemistry. First, as vanadate, it competes with phosphate at the active sites of phosphate transport proteins, phosphohydrolases, and phosphotransferases. Second, as vanadyl it competes with other transition metal ions for binding sites on metalloproteins and for small ligands such as adenosine triphosphate (ATP). Third, it participates in redox reactions within the cell, particularly with substances that can reduce vanadate nonenzymatically, such as glutathione.

A few of the more thoroughly investigated pharmacological effects of vanadium are described briefly. Vanadium inhibits Na^+/K^+ -ATPase, an enzyme involved in ATP's phosphorylation of the carrier protein for sodium ions by permitting the transport of the ions against a concentration gradient. Vanadate is known to inhibit the enzyme by binding to its ATP hydrolysis site. Vanadate has been shown to form ternary complexes with myosin and ADP to inhibit interactions with actin [13].

Vanadium, as vanadate, is believed to stimulate adenylate cyclase by promoting an association of an otherwise inactive guanine nucleotide regulatory protein (G protein)

with the catalytic unit of the enzyme [14]. Adenylate cyclase catalyzes the formation of cyclic 3',5'-adenosine monophosphate (cAMP) from ATP. Cyclic AMP then stimulates protein kinases, which catalyze the phosphorylation of various enzymes and other cellular proteins in cytoplasm, membranes, mitochondria, ribosomes, and the nucleus. The phosphorylation is nearly always stimulatory, and it results secondarily from the hormone-induced stimulation of adenylate cyclase. This relationship is the basis for cAMP's putative role as a second messenger of hormone action.

The effect of vanadate on the transport of amino acids across the intestinal mucosa exemplifies both its inhibitory effect on Na^+/K^+ -ATPase and its stimulation of adenylate cyclase. At higher concentrations, vanadate inhibits the mucosal-to-serosal flux of alanine, commensurate with a decrease in Na^+/K^+ -ATPase function. However, at a lower concentration (too low to affect Na^+/K^+ -ATPase), it stimulates alanine transport; this shift is attributable to an increase in adenylate cyclase activity and cAMP formation [15].

Vanadium, as vanadate and vanadyl, mimics the action of insulin. Vanadium stimulates glucose uptake into cells, enhances glucose metabolism, and inhibits catecholamine-induced lipolysis in adipose tissue [16]. The enhancement in glucose uptake occurs through improved translocation of glucose transporter GLUT4 to cell membranes [17,18]. Vanadium also stimulates glycogen synthesis in the liver and inhibits gluconeogenesis. Insulin works by binding to insulin receptors, which span the lipid membranes of cells. Insulin binding to cell membrane receptors results in tyrosine-specific, protein kinase–stimulated phosphorylation of tyrosine residues on the receptor. Serine and threonine phosphorylation of the receptor also may occur. The phosphorylation leads to multiple cascades of reactions. Vanadium is thought to mimic insulin function; however, vanadate phosphorylates tyrosyl residues not in the insulin receptor, but in cytosolic protein kinases and in non-insulin receptor protein kinases in the plasma membrane [9]. Activation of the cytosolic protein tyrosine kinases affects glucose and lipid metabolism, while activation of the plasma membrane protein tyrosine kinases triggers phosphatidylinositol 3-kinase, inhibits lipolysis, and stimulates glucose uptake [10]. Vanadate also has been shown to inhibit protein tyrosine phosphatase, thereby prolonging the activity of phosphorylated enzymes and enhancing the insulin-signalling pathway [17–19]. Evidence also exists that vanadium inhibits the activity of other enzymes such as glucose 6-phosphatase, fructose 2,6-biphosphatase, and acidic and alkaline phosphatase. In rats, sodium vanadate also appears to exert an insulinotropic effect by stimulating the release of insulin from islet cells [20].

Doses of 100–300 mg vanadyl sulfate or sodium metavanadate have used in clinical trials with people with type 2 diabetes [21–26]. Vanadium improved insulin sensitivity and thereby reduced serum glucose concentrations and hemoglobin A1c. Gluconeogenesis and serum lipid concentrations

were also reduced. Vanadium supplementation in diabetic rats diminished hyperglycemia, as well as polyuria and glucosuria [17,25]. See the article by Thompson [26] for a review of vanadium compounds in treating diabetes.

Although vanadate's chemical similarity to phosphate accounts in large part for its biochemical action, vanadium (as the vanadyl cation) also can substitute for other metals such as zinc, copper, and iron in metalloenzyme activity. Studies examining vanadium deficiency have suggested that the element is associated with iodine metabolism, thyroid gland function, or both [27]. Controlled depletion of vanadium has been reported to adversely affect growth rate, perinatal survival, physical appearance, hematocrit, and other manifestations in various animal species [28].

EXCRETION

Renal excretion is the major route for the elimination of absorbed vanadium [29]. Amounts of vanadium in the urine are generally <0.8 $\mu\text{g}/\text{L}$ urine. Urinary metabolites include vanadium diascorbate and vanadyl transferrin complex [30]. In addition to losses in the urine, small amounts (~1.0 ng/g) of vanadium are excreted in the bile.

RECOMMENDED INTAKE, TOXICITY, AND ASSESSMENT OF NUTRITION

The human requirement for vanadium is not established, although 10 $\mu\text{g}/\text{day}$ has been suggested as meeting the requirement [31]. A tolerable upper intake level of 1.8 mg elemental vanadium per day has been established [32]. Toxicity has been shown in humans with intakes above ~10 mg. Toxic manifestations include green tongue (from deposition of green-colored vanadium in the tongue), diarrhea, gastrointestinal cramps, disturbances in mental function, hypertension, and renal toxicity [1,12,32].

The techniques most commonly used for assessment are neutron activation analysis and flameless atomic absorption spectrophotometry; spectrophotometry is the practical choice of most analytic laboratories. Plasma or serum vanadium concentrations in healthy adults are low, with concentrations <0.4 mg/L and often 0.001 mg/L , and with supplementation range from about 1 to >500 ng/mL [3,24].

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Cobalt

Little evidence exists that cobalt plays a role in human nutrition other than its being a part of vitamin B₁₂ (cobalamin). Although ionic cobalt can substitute for other metals in metalloenzyme activity in vitro, no evidence exists that it acts in that capacity in vivo. In this respect, the metal is unique among the essential trace elements, in that the requirement in humans is not for an ionic form of the metal but for a preformed metallovitamin that cannot be synthesized from dietary metal. Therefore, it is the vitamin B₁₂ content of foods and diet, rather than the ionic cobalt present, that is important in human nutrition.

There have been reports regarding the dependency of certain enzymes on cobalt as an activator or on the metal's ability to substitute for other metal ion activators. Cobalt, in the form of Co²⁺, for example, appears to regulate the activity of certain phosphoprotein phosphatases, such as casein and phosvitin phosphatases [1,2]. In another study on phosphoprotein phosphatases, only Co²⁺ and Mn²⁺ could reactivate enzymes inactivated by ATP, ADP, and PP_i, with cobalt being significantly the more potent reactivator [3]. Cobalt, along with Mn²⁺ and Ni²⁺, can also substitute for Zn²⁺ in the metalloenzymes, angiotensin-converting enzyme [4], carboxypeptidase [5], and carbonic anhydrase [6].

The reader is cautioned against interpreting such findings as implying the possible essentiality of ionic cobalt. Deprivation studies in animals have produced no evidence that the metal is a requirement for these enzymes in vivo.

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Body Fluid and Electrolyte Balance

Water Distribution in the Body

Maintenance of Fluid Balance

Osmotic Pressure
Filtration Forces

The Kidney's Role

Maintenance of Electrolyte Balance

Sodium
Chloride
Potassium
Calcium and Magnesium

Acid-Base Balance: The Control of Hydrogen Ion Concentration

Acid-Base Buffers

Respiratory Regulation of pH

Renal Regulation of pH

Summary

PERSPECTIVE

Fluid Balance and the Thermal Stress of Exercise

Chapter 1, in particular, and the subsequent chapters dealing with nutrient metabolism emphasize the specialized nature of cells comprising the organ systems of the body. Despite the great diversity of specialized cellular functions, the composition of the body fluids (the internal environment) enveloping the cells remains relatively constant under normal conditions. This constant composition, or **homeostasis**, of the internal environment is necessary for optimal activity of the cells. It is maintained by homeostatic mechanisms involving most of the body's organ systems, the most important of which are the circulatory, respiratory, and renal excretory systems as well as the central nervous system (CNS) and the endocrine regulation system. Many minor disturbances inevitably occur in water distribution, electrolyte balance, and pH of the body fluids during metabolism. As disturbances arise, compensatory mechanisms of the regulatory organs make appropriate corrections to maintain homeostasis.

Water Distribution in the Body

Water accounts for about 60% of the total body weight in a normal adult, making it the most abundant constituent of the human body. In terms of volume, the total body water in a man of average weight (70 kg) is roughly 42 L. Water provides the medium for the solubilization and passage of a multitude of nutrients, both organic and inorganic, from the blood to the cells and for the return of metabolic products to the blood. It also serves as the medium in which the vast number of intracellular metabolic reactions takes place.

Total body water can theoretically be compartmentalized into two major reservoirs: the intracellular compartment, which includes all water enclosed within cell membranes, and the extracellular compartment, which includes all water external to cell membranes. Of the 42 L of total body water, the intracellular and extracellular compartments account for about 28 L and 14 L, respectively. The anatomic extracellular water is functionally subdivided into the plasma (the cell-free, intravascular water compartment) and the interstitial fluid (ISF). The ISF directly bathes the extravascular cells and provides the medium for the passage of nutrients and metabolic products back and forth between the blood and cells. In addition, potential spaces in the body (i.e., pericardial, pleural, peritoneal, and synovial) that are normally empty except for a small volume of viscous lubricating fluid must be considered part of the ISF compartment. The body water compartment volumes for a 70 kg man are summarized in Table 14.1.

Table 14.1 Fluid Compartment Values

	Percentage of Body Weight	Percentage of Total Body Water	Volume (L) in 70 kg Man
Total body water	60	—	42
Extracellular water	20	33	14
Plasma	5	8	3.5
Interstitial fluid	15	25	10.5
Intracellular water	40	67	28

The fraction of total body weight that is water and the percentage of total body water that is extracellular or intracellular do not remain constant during growth. Expressed as a percentage of body weight, total body water decreases during gestation and early childhood, reaching adult values by about 3 years of age. During this time, the extracellular water (as percentage of body weight) decreases while the intracellular water (as percentage of body weight) increases (Table 6.8).

Maintenance of Fluid Balance

Most of the daily available water enters by the oral route as beverages and as liquids contained in foods. A relatively small amount of water is formed within the body as a product of metabolic reactions. These two sources together account for a daily intake of about 2,500 mL fluid, of which the oral route contributes about 2,300 mL, or >90%.

The routes by which water is lost from the body can vary according to environmental and physiological conditions, such as ambient temperature and extent of physical exercise. At an ambient temperature of 68°F, about 1,400 mL of the 2,300 mL taken in is normally lost in the urine, 100 mL in the sweat, and 200 mL in the feces. The remaining 600 mL leaves the body as insensible water loss, so called because the subject is not aware of the water loss as it is occurring. Evaporation from the respiratory tract and diffusion through the skin are examples of insensible water loss.

OSMOTIC PRESSURE

One of the more important factors determining the distribution of water among the water compartments of the body is **osmotic pressure**. When a membrane permeable to water but impermeable to solute particles separates two fluid compartments of unequal solute concentrations, a net movement of water takes place through the membrane from the solution with higher water (lower solute) concentration toward the solution with lower water (higher solute) concentration. In other words, water moves from the more dilute solution to the more concentrated solution. The movement of water across a semipermeable membrane is called **osmosis**.

Osmosis can be blocked by applying an external pressure across the membrane in the opposite direction to the water flow. The amount of pressure required to exactly oppose osmosis (i.e., water movement) into a solution across a semipermeable membrane separating the solution from pure water is the osmotic pressure of the solution.

The theoretic osmotic pressure of a solution is proportional to the number of solute particles per unit weight of solvent. This concentration is expressed in terms of the osmolality, or osmoles of solute particles per kg of solvent (osm/kg). One mole of a nonionic solute, such as glucose or urea, is the same as 1 osm, but 1 mol of a solute that dissociates into two or more ions is equivalent to 2 osm or more. For example, 1.0 mol of sodium chloride equals 2.0 osm because of its dissociation into sodium and chloride ions. The theoretic osmotic pressure presupposes that the solute particles are unable to pass freely through the membrane. When the membrane is permeable to a solute, it does not contribute to the actual, or *effective*, osmotic pressure. The higher the permeability of a membrane is to a solute, the lower the effective osmotic pressure of a solution of that solute at a given osmolality. As an example, cell membranes are much more permeable to a nonionic substance such as urea than to sodium and chloride ions. Therefore, the effective osmotic pressure of a solution of urea across the cell membrane would be much less than that of a solution of sodium chloride of the same osmolality.

The term *osmolarity* is sometimes encountered in the expression of osmotic pressure. Osmolarity is similar to osmolality, but osmolarity denotes the concentration of solute particles in a designated amount of solution (1 liter). The concentration of a solution is expressed in terms of moles per liter of solution. Osmolarity is a similar weight-per-volume expression of concentration as molarity. Osmolality, the more accurate term, refers to solute concentration on a weight (osmoles) per weight (kg) of solvent basis (weight of solute to weight of solvent). Specifically, it is the moles of solute particles per kilogram of solvent. The expression of osmoles per kg of solvent provides a constant ratio of solute particles to molecules of solvent that is independent of temperature (aqueous solutions expand as they are heated). Osmolality is less convenient to use and calculate than osmolarity as a unit of concentration, but has the advantage of maintaining a constant ratio between the moles of solute particles and moles of solvent. In dilute aqueous solutions, as found in the human body, only a small numerical difference exists between the two values.

The effective osmotic pressure of plasma and interstitial fluid across the capillary endothelium that separates them is caused mainly by macromolecules, such as proteins, that cannot permeate the endothelium. Protein concentration is much higher in the plasma than in the interstitial fluid, conferring on the plasma a relatively high osmotic pressure, or water-attracting property. Proteins and other macromolecules too large to traverse the capillary endothelium

are sometimes called **colloids**, and the osmotic pressure attributed to them is appropriately termed the *colloid osmotic pressure*.

FILTRATION FORCES

Water distribution across the capillary endothelial surface is controlled by the balance of forces that tend to move water from the plasma to the interstitial fluid (filtration forces) and forces that move water from the interstitial fluid into the plasma (reabsorption forces). The major filtration force in the capillaries is hydrostatic pressure (P_{pl}) caused by the pumping of the heart. A much weaker filtration force is the ISF colloid osmotic pressure (II_{isf}); this force is weak because of the negligible concentration of protein in the ISF. Another weak filtration force is a small, negative ISF hydrostatic pressure (P_{isf}). The major reabsorption force countering the filtration forces is the plasma osmotic pressure (II_{pl}), which is approximately 28 mm Hg.

At the arteriolar end of the capillaries, the average values of these forces are P_{pl} (hydrostatic pressure), 25 mm Hg; II_{isf} (colloid osmotic pressure), 5 mm Hg; P_{isf} (interstitial hydrostatic pressure), -6 mm Hg; II_{pl} (plasma osmotic pressure), 28 mm Hg. The net result of these four forces can be described by Starling's equation:

$$\text{Filtration pressure} = (P_{pl} + II_{isf}) - (II_{pl} + P_{isf})$$

Substituting the average values, we have

$$\begin{aligned}\text{Filtration pressure} &= (25 + 5) - (28 + (-6)) \\ &= (25 + 5) - (28 - 6) \\ &= 30 - 28 + 6 \\ &= 8 \text{ mm Hg}\end{aligned}$$

This positive filtration pressure indicates that a net filtration of water from the plasma to the ISF occurs at the arteriolar end of the capillaries. When filtration pressure is negative, a net reabsorption of water from the ISF to the plasma occurs. This situation exists at the venule end of the capillaries, where P_{pl} is substantially reduced while the concentration of plasma protein, and therefore II_{pl} , correspondingly increases. The net effect of these forces on the water distribution between plasma and ISF along the course of the capillary is shown in Figure 14.1.

From what you have read to this point, you should understand that osmotic pressure, together with proper intake of fluids and their output by body mechanisms, is a very important factor in maintaining fluid balance and compartmentalization. The body's extracellular water volume, for example, is determined mainly by its osmolarity. The osmolarity, in turn, acts as the signal to the regulatory factors responsible for maintaining fluid homeostasis. The regulation of extracellular water osmolarity and volume is largely the responsibility of the hypothalamus, the renin-angiotensin-aldosterone system, and the kidney.

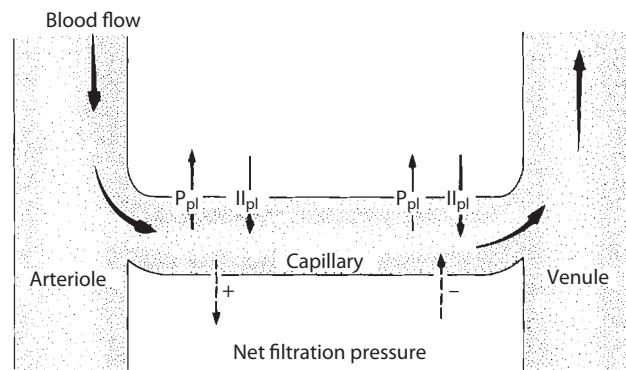


Figure 14.1 Starling's hypothesis of water distribution between plasma and interstitial fluid compartments. The relative magnitudes of the pressures, P_{pl} (plasma hydrostatic pressure) and II_{pl} (plasma osmotic pressure), are represented by the thickness of their respective arrows. There is a positive net filtration pressure at the arteriolar end of the capillary and a negative net filtration pressure at the venule end.

The Kidney's Role

The functional unit of the kidney is the nephron. Each kidney contains about 1 to 1.5 million nephrons. The five components of the nephron are the Bowman's capsule, proximal convoluted tubule, loop of Henle, distal convoluted tubule, and collecting duct. The excretion process starts in the Bowman's capsule, the blind, dilated end of the renal tubule, which encapsulates a tuft of about 50 capillaries linking the afferent (flowing into the capsule) and efferent (flowing from the capsule) arterioles that surround the tubule segments after they leave the capsule. The capillary network in the Bowman's capsule is called the glomerulus, and it accounts for the particularly rich blood supply that the kidney enjoys. An estimated 25% of the volume of blood pumped by the heart into the systemic circulation is circulated through the kidneys, a particularly significant situation in view of the fact that the kidneys constitute only about 0.5% of total body weight. The major components of the nephron are shown schematically in Figure 14.2.

The capillaries in the glomerular network have large pores and act as a filter in removing water and other substances, including electrolytes, glucose, amino acids, and metabolic waste products, from plasma. The capillaries of the glomerulus are 100 to 400 times more permeable to water and dissolved solutes than are the capillaries of skeletal muscle. The filtered substances make up what is known as the glomerular filtrate. In the absence of disease, no blood cells (or proteins that exceed a molecular weight of about 50,000 daltons) normally enter the glomerular filtrate, because their larger size prevents them from passing through the pores of the capillary endothelium.

Each segment of the tubules is functionally distinct in its permeability to water and the solutes of the glomerular filtrate. The tubular segments are surrounded by a network

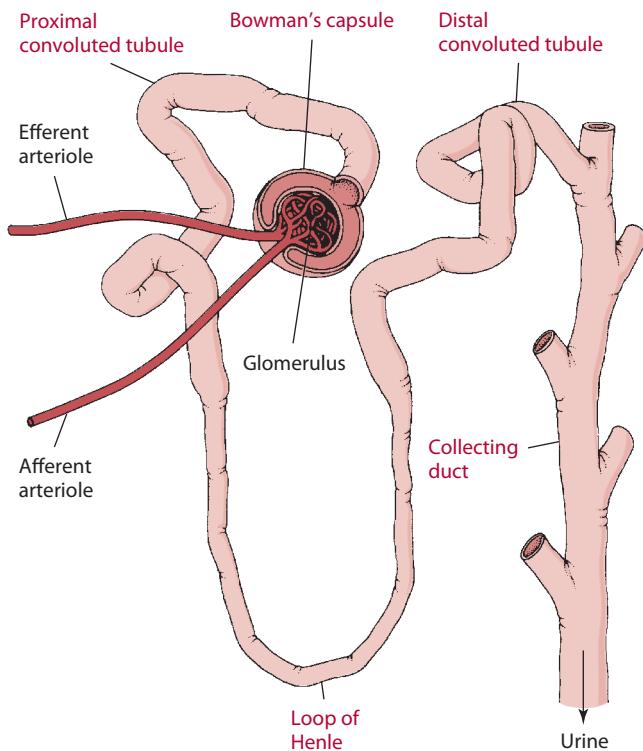


Figure 14.2 A schematic representation of the major components of the nephron.

of capillaries into which glomerular filtrate materials can be selectively reabsorbed into the bloodstream as a salvage mechanism. These peritubular capillaries may also secrete certain substances from the blood into the renal tubule. The removal of potentially toxic waste products, a major function of the kidneys, is accomplished through the formation of urine. Urine formation involves three basic processes:

- *filtration*, through which the glomerular filtrate is formed
- *reabsorption* of selected filtrate substances into the bloodstream
- *secretion* of materials into the tubules from the surrounding capillaries

Through these same processes, the kidneys are able to regulate fluid and electrolyte homeostasis for the proper functioning of cells throughout the body. In healthy people, the kidneys are highly sensitive to fluctuations in diet and beverage intake. They compensate for the variation in fluid and electrolyte intake by varying the volume and consistency of the urine. The glomerular capillaries differ from other capillaries in the body in that the hydrostatic pressure within them is approximately three times greater than in other capillaries. As a result of this high pressure, substances are filtered through the semipermeable membrane into the Bowman's capsule at a rate of about

130 mL/minute. This filtration rate amounts to over 187 liters of filtrate formed per day, yet only about 1,400 mL of urine is produced during this time. This difference in the volume filtrate and urine formation means that <1% of the filtrate is excreted as urine, and the remaining 99% is reabsorbed into the blood. The details of process by which urine is formed in the kidneys are beyond the scope of this book and can be obtained from a general physiology textbook such as Fox's *Human Physiology* [1]. An overview is provided here, with emphasis on the hormonal and enzymatic regulation.

You have read that the hypothalamus, the renin-angiotensin-aldosterone system, and the kidney are responsible for maintaining extracellular fluid volume and osmolarity. Actually, the three work in concert because the hypothalamic hormone, antidiuretic hormone (ADH, also called vasopressin), and aldosterone, produced in the adrenal cortex, exert their effects through the kidney.

Antidiuretic hormone is produced in the supraoptic nucleus of the hypothalamus but is stored in and secreted by the posterior pituitary gland. ADH is a potent water-conserving hormone. It increases the water permeability of the distal convoluted tubule and the collecting duct, thereby facilitating the reabsorption of water into the peritubular capillaries. Water is never "actively transported"; it moves across membranes because of changes in osmotic pressure. In the ascending limb of the loop of Henle, sodium, potassium, and chloride ions are actively pumped into the thick portion of the loop of Henle. Then Na^+ is actively pumped into the interstitial fluid, and the Cl^- ion follows. The potassium ion passively diffuses back into the glomerular filtrate. This process makes the interstitial fluid very hypertonic. The walls of the ascending limb of the loop of Henle are not permeable to water. Antidiuretic hormone (ADH) binds to receptors on the membrane of the collecting duct. Through the use of the second messenger cAMP, aquaporins (water channels) fuse in the membrane to make it more permeable to water. The water is moved into the capillaries and returned to general circulation. When ADH is present, more water is reabsorbed than when it is absent. ADH promotes Na^+ retention and K^+ excretion. The ADH is secreted into the circulation from the pituitary, triggered by increased extracellular water osmolarity or by decreased intravascular volume. The hypothalamic response to high extracellular fluid osmolarity is attributed to shrinkage of neurons within the gland, caused by the movement of water out of the neurons into the higher osmotic interstitial fluid. This shrinkage then acts as the signal to the posterior pituitary to release the hormone.

Aldosterone is a major factor in controlling sodium ion retention and potassium ion excretion. Several different substances influence the release of aldosterone, according to their plasma concentration. These substances are listed here and discussed again in the following section on maintenance

of electrolyte balance. Listed in decreasing order of their potency in stimulating aldosterone release, they are:

1. Increased angiotensin II. This potent polypeptide hormone participates in the renin-angiotensin pathway of aldosterone stimulation. It reacts with receptors on adrenal cell membranes, stimulating the synthesis and release of aldosterone.
2. Decreased atrial natriuretic peptide (ANP). ANP is a peptide hormone synthesized in atrial cells and released in response to increased arteriolar stretch, which indicates elevated blood pressure. It functions in opposition to aldosterone in that it inhibits sodium reabsorption in the kidney and thereby promotes sodium excretion [2].
3. Increased potassium concentration.
4. Increased ACTH.
5. Decreased sodium.

Angiotensin II is particularly important in stimulating aldosterone release. Therefore, this section describes the renin-angiotensin-aldosterone system in greater detail. Renin is a proteolytic enzyme synthesized, stored, and secreted by cells in the juxtaglomerular (near or adjoining the glomerulus) apparatus of the kidney. Renin secretion is stimulated by decreased renal perfusion pressure that is sensed by the distention receptors and baroreceptors within the juxtaglomerular apparatus. Renin hydrolyzes angiotensinogen (a freely circulating protein synthesized by the liver) to angiotensin I, an inactive decapeptide. Angiotensin I is then acted on by a second proteolytic enzyme, angiotensin-converting enzyme (ACE), synthesized in vascular endothelial cells (particularly those in the blood vessels of the lung), producing the potent octapeptide angiotensin II. Angiotensin II then interacts with specific receptors on adrenal cortical cells, leading to the release of aldosterone.

Let's review very briefly the mechanism of action of angiotensin II in increasing the synthesis and release of aldosterone from the adrenal cortex. Stimulatory signals resulting from polypeptide hormone-receptor interactions generally follow one of two major routes. One route operates through an accelerated synthesis of cAMP, with a consequent increase in protein kinase activity. The second route involves signals mediated by hydrolytic products of phospholipids and by increased intracellular calcium concentrations. It is the second of these mechanisms that applies in the case of angiotensin II action.

A sequential cascade of reactions follows the interaction of angiotensin II with its receptor, involving G proteins, phospholipase C, and inositol triphosphate. Phospholipase C raises intracellular Ca^{2+} concentration by increasing Ca^{2+} conductance through Ca^{2+} channels, and inositol triphosphate releases Ca^{2+} from its storage in the endoplasmic reticulum. The elevated concentration of intracellular

Ca^{2+} stimulates appropriate synthetic enzymes, mediated through the Ca^{2+} -binding protein calmodulin [3]. This interaction results in increased synthesis and release of aldosterone. Calmodulin is present in all eukaryotic cells. Figure 11.5 illustrates this type of hormonal mechanism.

The sequence of events that comprise the renin-angiotensin-aldosterone system is illustrated in Figure 14.3. Angiotensin II can be hydrolyzed further to angiotensin III (process not shown in the figure) by the hydrolytic removal of an aspartic acid residue by a plasma aminopeptidase. Angiotensin III is also physiologically active. In fact, it has been observed to be more potent than angiotensin II in its aldosterone-stimulating ability. However, the plasma concentration of angiotensin III is considerably less than that of angiotensin II, and therefore its contribution to maintaining fluid balance is less dramatic. In addition to its role in conserving body water through aldosterone action, angiotensin II is a potent vasoconstrictor, reducing the glomerular filtration rate and therefore the filtered load of sodium. Also, recall that angiotensin II stimulates the hypothalamic thirst center and the release of ADH, both of which increase body water volume. Figure 14.4 illustrates the central role of the hypothalamus and the action of angiotensin II in the hormonal regulation of fluid homeostasis.

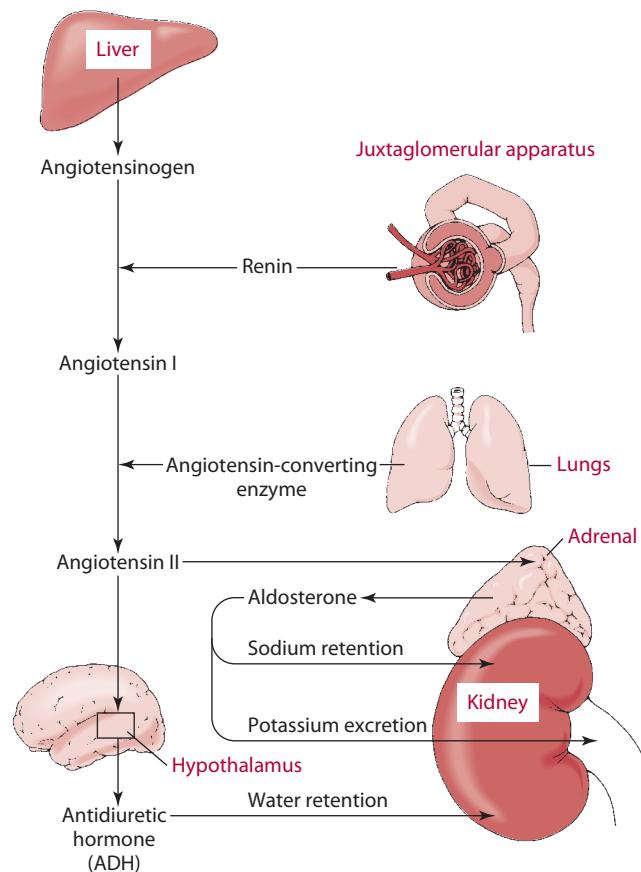


Figure 14.3 The renin-angiotensin-aldosterone system, illustrating the cooperation of kidneys, liver, lungs, adrenals, and hypothalamus in this mechanism of fluid homeostasis.

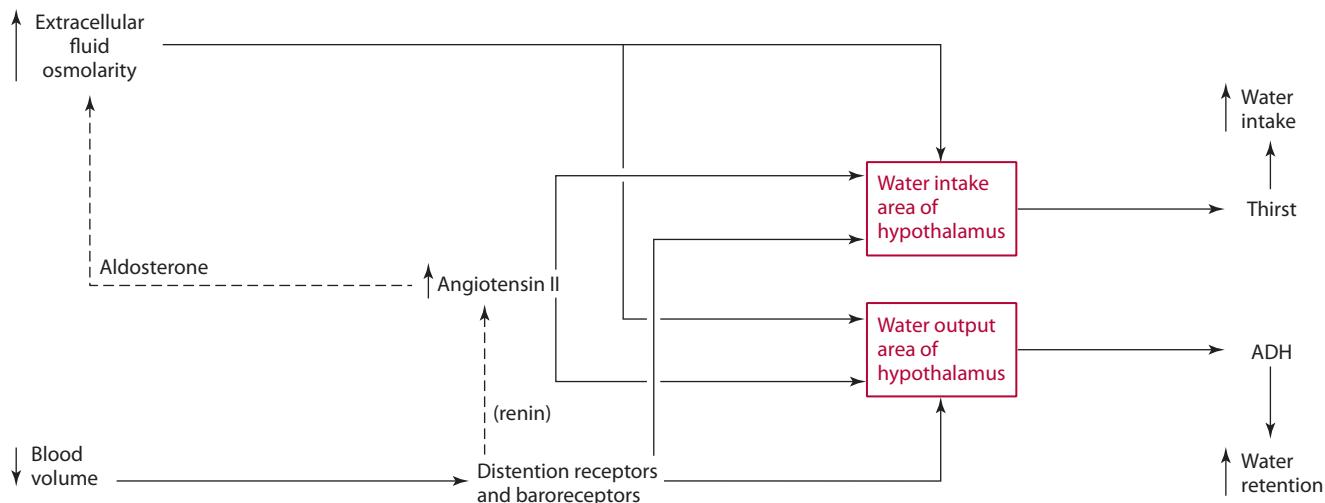


Figure 14.4 A summary of the mechanisms by which fluid homeostasis is maintained. Water depletion stimuli such as increased extracellular fluid osmolarity or decreased blood volume can stimulate the hypothalamus either directly or through the production of angiotensin II, formed by the action of the renal protease renin. The renin-angiotensin-aldosterone system (shown by dashed arrows) increases extracellular fluid osmolarity by promoting renal tubular reabsorption of sodium.

The mechanism aldosterone involves the transcription and translation of new proteins, which may be Na^+ channels in the luminal membrane, certain mitochondrial enzymes, or Na^+/K^+ -ATPase [1]. Evidence that protein induction is indeed a part of the mechanism of aldosterone action is provided by the fact that actinomycin D and puromycin, which are inhibitors of protein synthesis, inhibit electrolyte balance regulation. By stimulating sodium reabsorption, aldosterone increases extracellular fluid osmolarity, thereby promoting fluid retention by the body through the hypothalamus-ADH mechanism already discussed. This potential for fluid retention is the reason that high-sodium diets are contraindicated for people whose fluid “balance” is already upset by excessive retention of water, as with hypertension and edema.

Increased extracellular fluid osmolarity or decreased blood volume therefore influences what is known as the water output area of the hypothalamus. The term *water output function* refers to the fact that, because of the resulting increase in ADH, renal tubular reabsorption of water increases and urine output decreases. However, these factors also stimulate the water intake area of the hypothalamus, resulting in the conscious sensation of thirst. A greater intake of water thus follows, resulting in a dilution of extracellular fluid and increased blood volume, which in turn reduces the release of ADH as fluid homeostasis is restored. The release of ADH and the induction of the thirst sensation in response to plasma osmolarity are illustrated graphically in Figure 14.5.

Alterations in food intake can profoundly affect water and electrolyte balance. During the initial days of a period of fasting, for example, renal excretion of sodium increases markedly, whereas prolonged fasting tends to conserve

sodium ions. Refeeding causes a marked retention of sodium, probably caused by the ingestion of carbohydrate. Consequently, a rapid regain of body weight follows, caused by an increase in total body water secondary to the stimulation of vasopressin and thirst by the rise in plasma osmolarity. These alterations in sodium and water balance as a result of early-phase fasting and refeeding account for weight loss and weight regain to a far greater extent than would be predicted from the changes in caloric balance [4].

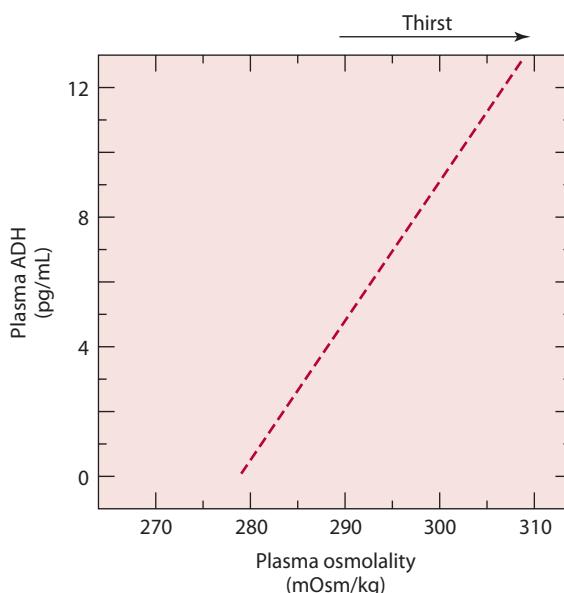


Figure 14.5 Relationship of plasma vasopressin to plasma osmolarity. The arrow indicates the plasma osmolarity at which the sensation of thirst is stimulated.

Maintenance of Electrolyte Balance

Electrolytes are the anions and cations that are distributed throughout the fluid compartments of the body. They are distributed in such a way that within a given compartment—the blood plasma, for example—electrical neutrality is always maintained, with the anion concentration exactly balanced by the cation concentration.

The cationic electrolytes of the extracellular fluid include sodium, potassium, calcium, and magnesium. These cations are electrically balanced by the anions chloride, bicarbonate, and protein, along with relatively low concentrations of organic acids, phosphate, and sulfate. The major electrolytes are listed in Table 14.2. Most are categorized nutritionally as macrominerals and, as such, are covered in Chapter 11 from the standpoint of absorption, function, dietary requirements, and food sources. The maintenance of pH and electrolyte balance, the focus of this chapter, is a responsibility that rests almost exclusively with the kidney.

All filterable substances in plasma—that is, all the plasma solutes except the larger proteins—freely enter the glomerular filtrate from the blood. Some of these substances are metabolic waste products and are excreted in the urine with little or no reabsorption in the tubules. However, most of the materials in the glomerular filtrate must be salvaged by the body. This salvage operation is accomplished through their tubular reabsorption by either active or passive mechanisms, or by tubular secretion. Active transport allows substances to pass across membranes against concentration gradients by the action of ATP-dependent membrane transport

systems (Chapter 1). Glucose is a prime example of a solute that can be actively transported across the tubular cells from the urine into the blood, even though the blood concentration of glucose normally is 20 times that of urine. Another group of solutes, including ammonium, potassium, and phosphate ions, occurs in relatively high concentration in urine compared to blood. These substances are transported from blood into the tubular cells, also against a concentration gradient. Passive transport, the simple diffusion of a material across a membrane from a compartment of higher concentration of the material to a compartment of lower concentration, is not energy demanding. This process, too, functions within the renal tubular cells. Now let's look at the renal regulation of several major electrolytes.

SODIUM

Sodium is freely filtered by the glomerulus. In a healthy person, nearly all (99.5%) of the sodium is reabsorbed. About 70% of the filtered sodium is reabsorbed by the proximal tubule, 15% by the loop of Henle, 5% by the distal convoluted tubule, and about 10% by the collecting ducts. Sodium is the major cation found in extracellular fluid.

Active reabsorption of sodium ions in the proximal tubule results in passive reabsorption of chloride ions, bicarbonate ions, and water. The accompanying transfer of the anions chloride and bicarbonate with the cation sodium is required to maintain the necessary electrical neutrality of the extracellular fluid, while the water transfer maintains normal osmotic pressure. Virtually all cells contain a relatively high concentration of potassium and a low concentration of sodium, whereas the blood plasma and most other extracellular fluids have high sodium and low potassium concentrations, as can be seen in Table 14.2. Clearly, energy must be expended to maintain this gradient across the cell membrane; otherwise, each ion would simply diffuse through the membrane until its intracellular and extracellular concentrations were the same. The gradient is maintained by the Na^+/K^+ -ATPase pump, which is described in Chapter 1. The Na^+/K^+ -ATPase pump is the mechanism by which the renal tubular cells “pump” sodium into the blood in exchange for potassium in such a way as to conserve sodium while allowing a constant loss of potassium in the urine.

Active reabsorption of sodium occurs in the distal convoluted tubule under the influence of aldosterone. The mechanism is highly selective for sodium ion and is accompanied by little water diffusion. This attribute makes it an important system for regulating extracellular fluid osmotic pressure. The increased retention of sodium by this mechanism also increases the osmolality of plasma and therefore is accompanied by water retention. When extracellular fluid osmotic pressure increases, tubular water reabsorption is stimulated through ADH release (Figure 14.4).

Table 14.2 Electrolyte Composition of Body Fluids

	Plasma (mEq/L)	Interstitial Fluid (mEq/LH ₂ O)	Intracellular Water (mEq/LH ₂ O)
Cations	153	153	195
Na ⁺	142	145	10
K ⁺	4	4	156
Ca ²⁺	5	(2–3)	3.2
Mg ²⁺	2	(1–2)	26
Anions	153	153	195
Cl [−]	103	116	2
HCO ₃ [−]	28	31	8
Protein	17	—	55
Others	5	(6)	130
Osmolarity (mosm/L)		294.6	294.6
Theoretic osmotic pressure (mm Hg)		5,685.8	5,685.8

CHLORIDE

The concentration of chloride in the extracellular fluid parallels that of sodium, and chloride generally accompanies sodium in transmembrane passage. However, recall that chloride reabsorption is passive in the proximal tubule. Chloride is probably reabsorbed actively in the ascending limb of the loop of Henle and the distal tubule.

POTASSIUM

Potassium is the chief cation of intracellular fluid, and maintaining a normal level is essential to the life of the cells. The healthy person maintains potassium balance by the daily excretion in the urine of an amount of the cation equal to the amount ingested, minus the small amount excreted in the feces and sweat. Potassium is freely filtered at the glomerulus, and its active tubular reabsorption occurs throughout the nephron, except for the descending loop of Henle. Only about 10% of the filtered potassium enters the distal tubules, which, along with the collecting ducts, are able to both secrete and reabsorb potassium. The distal tubule is the site at which changes in the amount of potassium excreted are achieved. Several mechanisms are involved in this control.

- The first of these mechanisms depends on the cellular potassium content. When a high-potassium diet is consumed, the concentration of potassium rises in cells, including the distal renal tubular cells, providing a concentration gradient that favors the secretion of the cation into the lumen of the tubule. This process results in an increase in potassium excretion.
- Another important mechanism in regulating potassium balance involves the hormone aldosterone, which, besides stimulating distal tubular reabsorption of sodium, simultaneously enhances potassium secretion at that site. The elevated plasma level of potassium directly stimulates the production and release of aldosterone from the adrenal cortex. Recall that another mechanism for effecting aldosterone release is through decreased renal perfusion pressure and the associated renin-angiotensin-aldosterone pathway.
- A third mechanism of renal conservation of potassium occurs in the collecting duct and involves potassium's active reabsorption coupled with the secretion of protons at that site [5]. The movement of K^+ into the cells of the collecting duct from the urine and the movement of H^+ in the opposite direction are catalyzed by an H^+/K^+ -activated adenosine triphosphatase (H^+/K^+ -ATPase), functioning similarly to the Na^+/K^+ -ATPase pump discussed previously.

CALCIUM AND MAGNESIUM

Tubular reabsorption of calcium is associated with the reabsorption of sodium and phosphate in the proximal

tubule, and reabsorption of all three ions, as well as fluid, occurs in parallel. Renal tubular reabsorption of calcium is closely linked to the action of parathyroid hormone (PTH) (Chapter 11). This hormone exerts parallel inhibition of the reabsorption of calcium, sodium, and phosphate in the proximal tubules. However, stimulation of the reabsorption of calcium in the distal tubules by PTH is markedly disproportionate to that of sodium and phosphate.

The major pathway of calcium excretion is the intestinal tract. Urinary excretion, about 150 mg/day for the average adult, amounts to only about 1% of that filtered by the glomerulus. The remaining 99% is effectively reabsorbed at proximal and distal tubular sites. Calcium balance is achieved largely by control of the intestinal absorption of the ion rather than by regulation of its urinary excretion. The percentage of ingested calcium absorbed decreases as the dietary calcium content increases, so the amount absorbed remains relatively constant. The slight increase in absorption that occurs with a high-calcium diet is reflected in an increased renal excretion of the cation.

The filtration of magnesium at the glomerulus and its subsequent active reabsorption through the tubular cells parallel the filtration and reabsorption of calcium.

Homeostatic regulation of the ions discussed is crucial to many body functions. For example, greatly decreased extracellular potassium levels (hypokalemia) produces paralysis, whereas elevated potassium levels (hyperkalemia) can result in cardiac arrhythmias. Excessive extracellular sodium (hypernatremia) causes fluid retention, and decreased plasma calcium (hypocalcemia) produces tetany (intermittent spasms of the muscles of the extremities) by increasing the permeability of nerve cell membranes to sodium. Magnesium deficiency is also associated with tetany.

Table 14.2 lists the fluid electrolytes and their approximate normal compartment concentrations. In terms of electrolyte balance only, the contribution of sodium to the body's total cation milliequivalents (mEq) is clearly quite large compared to that of potassium, calcium, and magnesium, and a correspondingly high percentage of anion milliequivalents is contributed by chloride and bicarbonate together. The concentration of these three major ions is used to calculate the so-called anion gap, a clinically useful parameter for establishing metabolic disorders that can alter electrolyte balance. The value is calculated by subtracting the measured anion (chloride + bicarbonate) concentration from the measured cation (sodium) concentration: Measured cations (Na^+) – measured anions ($Cl^- + HCO_3^-$) = anion gap. Under normal conditions, the value is about 12 mEq/L, but it may range from 8 to 18 mEq/L. Deviation from a normal anion gap is most commonly associated with increases or decreases in the concentration of certain unmeasured anions such as proteins, organic acids, phosphate, or sulfate. For example, the production of excessive amounts of organic acids, such as

would occur in lactic acidosis or ketoacidosis, increases the unmeasured anion concentration at the expense of the measured anion bicarbonate that is neutralized by the acids. Such a condition would therefore cause a greater anion gap.

Considering the effect of plasma osmolality on water intake and retention, it is logical that should sodium ion accumulate in the body water for any reason, a concomitant rise in blood pressure (essential hypertension) would result. Clinical evidence for this correlation is provided by the hypertension experienced by patients with adrenal adenomas, whose high levels of aldosterone cause excessive retention of sodium. An apparent causal relationship also exists between dietary intake of sodium (as sodium chloride) and the etiology of hypertension, as suggested by studies conducted through one or more of the following designs:

- relating salt consumption to the prevalence of hypertension
- tracking development of hypertension in animals fed high-salt diets
- measuring response of hypertensive patients fed low-salt diets

An abundance of reported observations deals with the positive correlation of salt intake and hypertension among societies that ingest salt to variable extents. Such observations have led to the generally accepted conclusion that the incidence of hypertension is predictable from average daily sodium intake. Also, convincing animal studies dating back to the 1950s have demonstrated a direct correlation between sodium chloride and hypertension. In spite of these findings, however, evidence of a cause-and-effect relationship among people in a normotensive population is scant. In fact, investigations on the effect on blood pressure of sodium chloride loading among normotensives have revealed no correlation between high salt intake and hypertension. Furthermore, among subjects with borderline essential hypertension, a low-sodium diet is minimally effective in lowering blood pressure. This lack of effect suggests that plasma sodium concentrations are unalterable if the homeostatic mechanisms controlling it are intact. It has become generally accepted that the differences between those who respond to sodium diet therapy and those who do not have a genetic foundation.

People who are salt-sensitive are called responders, and those who show salt insensitivity are labeled nonresponders. The condition of nonresponders who have essential hypertension does not improve with a low-salt diet. Likewise, normotensive nonresponders can consume as much as 4,600 mg sodium daily (somewhat higher than the sodium intake of the typical Western diet) without risk. Among genetically disposed (i.e., salt-sensitive) people, a comparable intake would likely favor the development of hypertension. For people in this population, a restriction of sodium intake to about 1,400 mg or less is recommended.

Although a genetic link to salt sensitivity is generally accepted, biochemical mechanisms of the condition are not clearly understood—though not for lack of relevant research. A literature review of the many investigations designed to explain the biochemical basis of salt sensitivity and nonsensitivity is available [6].

In summary, sodium's role in hypertension remains controversial. It likely does not function alone in the etiology of the disease, and it may be a contributing factor only in the wake of other biochemical disturbances. The involvement of other cations such as calcium, magnesium, potassium, and cadmium cannot be overlooked [6]. Potassium intake has been linked to a reduction in blood pressure, especially in people on high-sodium diets. Although the mechanism remains unknown, potassium may affect natriuresis, baroreflex sensitivity, catecholamine function, or the renin-angiotensin-aldosterone system [7].

Acid-Base Balance: The Control of Hydrogen Ion Concentration

The hydrogen ion concentration in body fluids must be controlled within a narrow range. In fact, its regulation is one of the most important aspects of homeostasis, because merely slight deviations from normal acidity can cause marked alteration in enzyme-catalyzed reaction rates in the cells. Hydrogen ion concentration can also affect both the cellular uptake and regulation of metabolites and minerals and the uptake and release of oxygen from hemoglobin.

The degree of acidity of any fluid is determined by its concentration of protons (H^+). The hydrogen ion concentration in body fluids is generally quite low: it is regulated at approximately 4×10^{-8} mol/L. Concentrations can vary from as low as 1.0×10^{-8} mol/L to as high as 1.0×10^{-7} mol/L, but values outside this range are not compatible with life. From these values, it is apparent that expressing H^+ in terms of its actual concentration is awkward. The concept of pH, which is the negative logarithm of the H^+ concentration, was devised to simplify the expression. It enables concentrations to be expressed as whole numbers rather than as negative exponential values:

$$pH = -\log [H^+]$$

Bracketed values symbolize concentrations. Throughout this discussion, this designation is used to signify concentrations of other substances besides protons. The pH of extracellular fluid, in which the H^+ concentration may be assumed to be approximately 4×10^{-8} mol/L, can therefore be calculated as follows:

$$pH = -\log (4 \times 10^{-8})$$

$$\text{or } pH = \log (1/(4 \times 10^{-8}))$$

$$\begin{aligned}
 (\text{dividing}) &= \log (0.25 \times 10^8) \\
 &= \log 0.25 + \log 10^8 \\
 (\text{taking logs}) &= -0.602 + 8 \\
 \text{pH} &= 7.4
 \end{aligned}$$

As the molar concentration of H^+ becomes smaller and the value of the negative exponent of 10 becomes larger, the pH correspondingly increases. Low acidity therefore denotes low H^+ concentration and high pH, whereas high acidity is associated with high H^+ concentration and low pH.

An acid, as it relates to fluid acid-base regulation, may be defined as a substance capable of releasing protons (H^+). The metabolism of the major nutrients continuously generates organic acids, which must be neutralized. Chapter 3 explains how lactic acid and pyruvic acid can accumulate in periods of oxygen deprivation, and Chapter 5 describes how fatty acids are released from triacylglycerols during lipolysis. Also, the acidic ketone bodies, acetoacetic acid and β -hydroxybutyric acid, can increase substantially during periods of prolonged starvation or low carbohydrate intake. Carbon dioxide, the product of the complete oxidation of energy nutrients, is itself indirectly acidic, because it forms carbonic acid (H_2CO_3) on combination with H_2O . Acidic salts of sulfuric and phosphoric acids are also generated metabolically from sulfur- or phosphorus-containing substances.

The term *acidosis* refers to a rise in extracellular (principally plasma) H^+ concentration (a lower pH) beyond the normal range. Abnormally low H^+ concentration, in contrast (i.e., high plasma pH), results in **alkalosis**. To guard against such fluctuations in pH, three principal regulatory systems are available:

- buffer systems within the fluids that immediately neutralize acidic or basic compounds
- the respiratory center, which regulates breathing and the rate of exhalation of CO_2
- renal regulation, by which either acidic or alkaline urine can be formed to adjust body fluid acidity

Acid-Base Buffers

A buffer is anything that can reversibly bind protons. In the body, a buffer is a chemical solution designed to resist changes in pH despite the addition of acids or bases. A buffer usually consists of a weak acid, which can be represented as HA, and its conjugate base (A^-). The conjugate base, therefore, is the residual portion of the acid following the release of the proton. The conjugate base of a weak acid is basic, because it tends to attract a proton and to regenerate the acid. Therefore, the dissociation of a weak acid and the reunion of its conjugate base and proton comprise an equilibrium system:



The equilibrium expression for this reaction, called the acid dissociation constant (K_a), is represented as

$$K_a = \frac{[\text{H}^+] [\text{A}^-]}{[\text{HA}]}$$

The equation can be rearranged to

$$[\text{H}^+] = \frac{K_a [\text{HA}]}{[\text{A}^-]}$$

Taking the negative logarithm of both sides of the equation gives

$$-\log [\text{H}^+] = -\log K_a - \log \frac{[\text{HA}]}{[\text{A}^-]}$$

These values become

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

This equation is referred to as the Henderson-Hasselbalch equation. It shows how a buffer system composed of a weak acid and its conjugate base resists changes in pH if strong acid or base is added to the system. For example, if the molar concentrations of the conjugate base and the acid are equal, then the ratio of $[\text{A}^-]$ to $[\text{HA}]$ is 1.0, and the logarithm of this ratio is 0, making the pH of the system equal to the $\text{p}K_a$ of the acid.

The $\text{p}K_a$, which is the negative logarithm of the acid dissociation constant (K_a), of any weak acid is a constant for that particular acid and simply reflects its strength (i.e., its tendency to release a proton). If a strong acid or a strong base is added to this system, the ratio of $[\text{A}^-]$ to $[\text{HA}]$ changes and therefore the pH changes, but only slightly. Suppose, for example, that both the conjugate base and the free acid are present at 0.1 mol/L concentrations, and suppose also that the $\text{p}K_a$ of the acid is 7.0. As shown above, if the ratio is 0.1:0.1, the pH is 7.0. The addition of enough hydrochloric acid (a strong acid) to the buffer in the example to make its final concentration 0.05 mol/L shifts the equilibrium to the left (to make HA). The 0.05 mol/L of H^+ (from the fully dissociated HCl) will combine with an equal molar amount of A^- to form HA. The new $[\text{A}^-]$ concentration therefore becomes 0.05 mol/L ($0.1 - 0.05$), and $[\text{HA}]$ is 0.15 mol/L ($0.1 + 0.05$). The logarithm of this new ratio (0.05:0.15, or 0.33) is -0.48 . Inserting this value into the Henderson-Hasselbalch equation, we can see that the pH decreases by only 0.48. In other words, the pH decreased from 7.0 to 6.52 by making the system 0.05 mol/L hydrochloric acid. In contrast, this same concentration of HCl in an unbuffered, aqueous solution would produce an acid pH between 1.0 and 2.0.

The physiologically important buffers that maintain the narrow pH range of extracellular fluid at ~ 7.4 are proteins and the bicarbonate (HCO_3^-)-carbonic acid (H_2CO_3) system. Proteins have the most potent buffering capacity among the physiological buffers, and, because of its high concentration in whole blood, hemoglobin is most important in this respect. The binding of oxygen to

hemoglobin is influenced by the pH of the blood. For the proper uptake and release of oxygen in the erythrocyte to occur, it is crucial for the pH regulation to be operating. As **amphoteric** substances (substances that possess both acidic and basic groups on their amino acid side chains), proteins are capable of neutralizing either acids or bases. For instance, the two major buffering groups on a protein are carboxylic acid ($\text{R}-\text{COOH}$) and amino ($\text{R}-\text{NH}_3^+$) functions, which dissociate as shown:

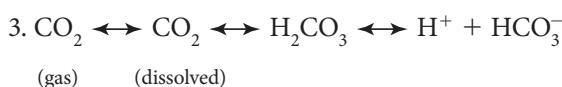
1. $\text{R}-\text{COOH} \leftrightarrow \text{R}-\text{COO}^- + \text{H}^+$
2. $\text{R}-\text{NH}_3^+ \leftrightarrow \text{R}-\text{NH}_2 + \text{H}^+$

At physiological pH, the carboxylic acid is largely dissociated into its conjugate base and a proton, so the equilibrium as shown is shifted strongly to the right. At that same pH, however, the amino group, being much weaker as an acid (a stronger base), is only weakly dissociated, and its equilibrium greatly favors the right-to-left direction. If protons, in the form of a strong acid, are added to a protein solution, they are neutralized by reaction 1, because their presence will cause a shift in the equilibrium toward the undissociated acid (right to left). Strong bases, as contributors of hydroxide (OH^-) ions, will likewise be neutralized because, as they react with the protons to form water, the equilibrium of reaction 2 (as illustrated) shifts to the right to restore the protons that were neutralized.

The bicarbonate–carbonic acid buffer system is of particular importance, because it is through this system that respiratory and renal pH regulation is exerted. This buffer system is composed of the weak acid, carbonic acid (H_2CO_3), and its salt or conjugate base, bicarbonate ion (HCO_3^-). The carbonic acid dissociates reversibly into H^+ and HCO_3^- :



The buffering capacity of this reaction arises from the fact that either added protons or added hydroxide ions will be neutralized by corresponding shifts in the equilibrium, similar to the carboxy-amino group buffering by proteins described earlier. The H_2CO_3 can be formed not only from the acidification of HCO_3^- , as shown in the right-to-left reaction above, but also from the reaction of dissolved CO_2 with water. Recall that CO_2 is formed as a result of total oxidation of the energy nutrients as well as various decarboxylation reactions. The gas diffuses from tissue cells into the extracellular fluids and then into erythrocytes, where its reaction with water to form H_2CO_3 is accelerated by the zinc metalloenzyme carbonic anhydrase. The overall reaction involving carbon dioxide, carbonic acid, and bicarbonate ion is as follows:



In the lungs, these equilibrium reactions are shifted strongly to the left in the circulating erythrocytes because of the release of protons from hemoglobin as hemoglobin acquires oxygen to become oxyhemoglobin. This shift allows the exhalation of carbon dioxide.

Normally, the ratio of the concentration of HCO_3^- to H_2CO_3 in plasma is 20:1, and the apparent pK_a value for H_2CO_3 is 6.1. Using the Henderson-Hasselbalch equation, we can show how a normal plasma pH of 7.4 results from these values:

$$\begin{aligned} \text{pH} &= \text{pK}_a + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \\ &= 6.1 + \log \frac{20}{1} \\ &= 6.1 + 1.3 \\ \text{pH} &= 7.4 \end{aligned}$$

Alterations in the 20:1 ratio of $[\text{HCO}_3^-]$ to $[\text{H}_2\text{CO}_3]$ clearly change the pH. The next section shows how respiratory and renal regulatory systems function to keep this ratio, and therefore the pH, relatively constant.

Respiratory Regulation of pH

If plasma levels of CO_2 rise, perhaps because of accelerated metabolism, more H_2CO_3 is formed. This reaction, in turn, causes a fall in pH as it dissociates to release protons (reaction 3). The elevated CO_2 itself, as well as the resulting increase in hydrogen ion concentration, is detected by the respiratory center of the brain, resulting in an increase in the respiratory rate. This hyperventilation increases CO_2 loss through the lungs substantially and therefore decreases the amount of H_2CO_3 . This mechanism increases the ratio of HCO_3^- to H_2CO_3 by reducing H_2CO_3 , thus elevating the pH to a normal value. Conversely, if plasma pH rises for any reason (because of either an increase in HCO_3^- or a decrease in H_2CO_3), the respiratory center is signaled accordingly and causes a slowing of the respiration rate. As CO_2 then accumulates, the H_2CO_3 concentration rises and the pH decreases.

Renal Regulation of pH

Although the intact respiratory system acts as an immediate (in minutes) regulator of the $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ system, long-term control (hours or days) is exerted by renal mechanisms. The kidneys regulate pH by controlling the secretion of hydrogen ions, by conserving or producing bicarbonate, and by synthesizing ammonia from glutamine to form ammonium ions. The secretion of

hydrogen ions occurs in conjunction with the tubular reabsorption of sodium ions through the mechanism of countertransport, an active process involving a common Na^+/H^+ carrier protein and energy sufficient to move the protons from the tubular cells into the tubule lumen against a concentration gradient of protons. In subjects on a normal diet, about 50 to 100 mEq of hydrogen ions are generated daily. Renal secretion of the protons is necessary to prevent a progressive metabolic acidosis. The renal tubules are not very permeable to bicarbonate ions because of the charge and the relatively large size of the ions. They therefore are reabsorbed by a special indirect process. The hydrogen ions in the glomerular filtrate convert filtered bicarbonate ions to H_2CO_3 , which dissociates into CO_2 and H_2O . The CO_2 diffuses into the tubular cell, where it combines with water, in a reaction catalyzed by carbonic anhydrase, to form H_2CO_3 . The relatively high tubular-cell pH allows the dissociation of the H_2CO_3 into HCO_3^- and H^+ , after which the bicarbonate reenters the extracellular fluid and the proton is actively returned to the lumen by the Na^+/H^+ carrier. The net result is to excrete an H^+ and to reabsorb a bicarbonate ion, even though it is not the same bicarbonate ion. These events, by which hydrogen ions are secreted against a

concentration gradient in exchange for sodium ions, and bicarbonate is returned to the plasma from the glomerular filtrate, are summarized in Figure 14.6.

The pH of the urine normally falls within the range of 5.5 to 6.5, despite the active secretion of hydrogen ions throughout the tubules. This pH is largely achieved by partial neutralization of the hydrogen ions by ammonia, which is secreted into the lumen by the tubular cells. Ammonia is produced in large amounts from the metabolic breakdown of amino acids. Although most of the nitrogen is excreted in the form of urea, some is delivered to the kidney cells in the form of glutamine. In the renal tubule cells, ammonia is hydrolytically released from glutamine by the enzyme glutaminase and is secreted into the urine (Chapter 6). Because it is a basic substance, ammonia immediately combines with protons in the collecting ducts to form ammonium ions (NH_4^+), which are excreted in the urine primarily as their chloride salts.

Should metabolic acidosis occur, such as in starvation or diabetes, urinary excretion of ammonia increases concomitantly to compensate. This increase occurs because the diminished intake and use of carbohydrate stimulate gluconeogenesis and therefore enhance excretion of ammonia, which is formed from the higher rate of amino acid catabolism.

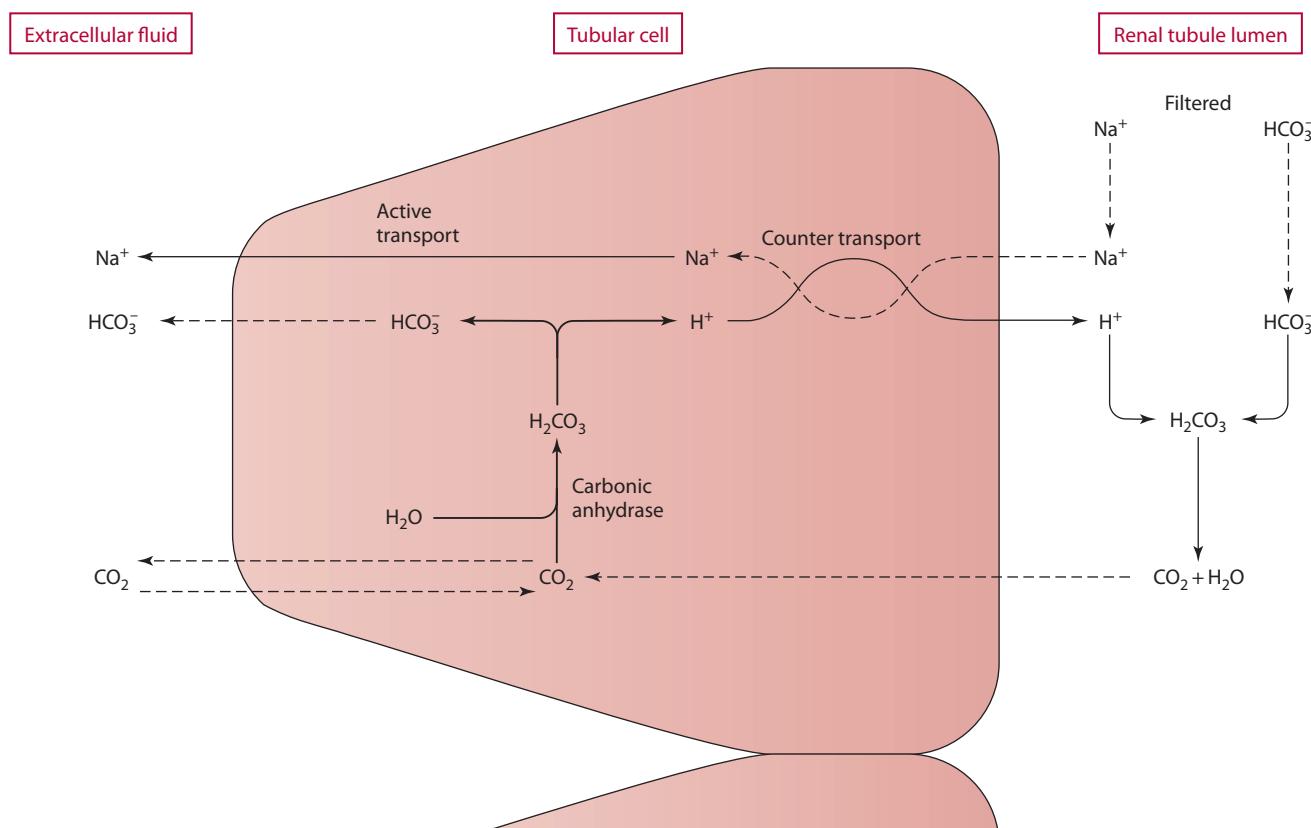


Figure 14.6 Renal tubular cell reactions illustrating the origin of and the active secretion of hydrogen ions in exchange for sodium ions, as well as the mechanism for tubular reabsorption of bicarbonate. Solid arrows indicate reactions or active transport, while dashed arrows signify diffusion.

Like respiratory regulation, renal regulation of pH is directed at maintaining a normal ratio of $[HCO_3^-]$ to $[H_2CO_3]$. In alkalosis, for example, in which the plasma ratio of HCO_3^- to H_2CO_3 increases as the pH rises above 7.4, a net increase occurs in the excretion of bicarbonate ions. This increase occurs because the high extracellular HCO_3^- concentration increases its filtration, while the relatively low concentration of H_2CO_3 decreases the secretion of H^+ . Therefore, the fine balance between HCO_3^- and H^+ that normally exists in the tubules no longer is in effect. Also, because no HCO_3^- ions can be reabsorbed without first reacting with H^+ (Figure 14.6), all the excess HCO_3^- passes into the urine, neutralized by sodium ions or other cations. In effect, therefore, HCO_3^- is removed from the extracellular fluid, restoring the normal ratio of HCO_3^- to H_2CO_3 and pH.

SUMMARY

Maintaining body fluids and electrolytes is vitally important for sound health and nutrition. Intracellular fluid provides the environment for the myriad of metabolic reactions that take place in cells. The interstitial fluid compartment of the extracellular fluid mass allows nutrients to migrate into cells from the bloodstream and metabolic waste products from the cells to return to the bloodstream. These fluids contain the electrolytes, dissolved minerals that have important physiological functions. Their concentrations and their intracellular and extracellular distribution must be precisely regulated, and the mechanism for achieving this regulation is exerted largely through the kidney. The homeostatic maintenance of fluid volume is also the responsibility of this organ.

Fluid volume control by the kidney is mostly hormone mediated. ADH, produced in the hypothalamus, stimulates the tubular reabsorption of water from the glomerular filtrate. Aldosterone, a product of the adrenal cortex, increases the reabsorption of sodium ions, which indirectly stimulate ADH release through the resulting rise in extracellular fluid osmotic pressure. Thirst centers in the brain, which respond to fluctuations in blood volume or extracellular fluid osmolality, are also important regulators of fluid balance by their influence on the amount of fluid intake.

The macrominerals sodium and potassium, and other ions of nutritional importance such as calcium, magnesium, and chloride, are freely filtered by the renal glomerulus but are selectively conserved by tubular reabsorption through active transport systems. Potassium is an example of a mineral that is regulated in part by tubular secretion into the filtrate. Secretion of the ion from the distal tubular cells increases as its concentration in those cells rises because of increased dietary intake. Potassium, like

In acidosis, the ratio of plasma HCO_3^- to H_2CO_3 decreases, meaning that the rate of H^+ secretion rises to a level far greater than the rate of HCO_3^- filtration into the tubules. As a result, most of the filtered HCO_3^- is converted to H_2CO_3 and reabsorbed as CO_2 (Figure 14.6), while the excess H^+ is excreted in the urine. As a consequence, the extracellular fluid ratio of $[HCO_3^-]$ to $[H_2CO_3]$ increases, as does the pH. The importance of the kidney in the homeostatic control of body water, as well as in electrolyte and acid-base balance, is emphasized in this chapter. The material is presented as a review of the principles involved in such control and of the effect of diet on fluid and electrolyte homeostasis. Although a detailed account of renal physiology is beyond the scope of this text, excellent sources that deal specifically with this subject are available [8,9].

sodium, is regulated by aldosterone. Elevated plasma potassium stimulates the release of aldosterone, which exerts opposing renal effects on the two minerals—enhanced reabsorption of sodium and an increase in potassium excretion. Normal physiological function depends on proper control of the body fluid acid-base balance.

Many metabolic enzymes have a narrow range of pH at which they function adequately, and these catalysts are intolerant of pH swings of more than several tenths of a unit from the average normal value of 7.4 for extracellular fluids. The plasma is well buffered, primarily by proteins and by the bicarbonate–carbonic acid system. However, conditions of acidosis or alkalosis can result in situations such as an overproduction of organic acids, as would occur in diabetes or starvation, or respiratory aberrations that may cause abnormal carbon dioxide ventilation. Therefore, restoration of normal pH may be necessary and is accomplished through compensatory mechanisms of the kidneys and lungs. These organs function to maintain a normal ratio of bicarbonate to carbonic acid. The bicarbonate concentration is under the control of the kidneys, which can either conserve the ion by reabsorbing it to a greater extent or increase its excretion, depending on whether the ratio needs to be decreased or increased to compensate for a pH disturbance. The carbonic acid value is controlled by the respiratory center. Its concentration can be increased or decreased by changes in the respiratory rate. Hyperventilation, for example, lowers the value by “blowing off” carbon dioxide, whereas a slowing of respiration retains carbon dioxide and therefore raises the carbonic acid level. From their effects on the bicarbonate:carbonic acid ratio, one can reason that hyperventilation can raise the pH and suppression of the respiratory rate can lower the pH in a compensatory manner.

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http://nkdep.nih.gov/patients/kidney_disease_information.htm

National Institutes of Health web site on kidney function and disease.

www.kidney.org/atoz

National Kidney Foundation. Provides information on kidney disease including nutritional aspects.

Fluid Balance and the Thermal Stress of Exercise

The discussion of sports nutrition in Chapter 7 centers on: (1) the selective use of energy-yielding substrates during exercise of varying durations and intensities and (2) how physical performance can be enhanced by maximizing substrate stores via the judicious intake of energy nutrients. Another very important dimension in the demands of sport and exercise is **thermoregulation**, the control of body temperature within a narrow range. A drop in deep body (core) temperature of 10°F and an increase of just 5°F above normal is tolerated, but fluctuation beyond this range can result in death. Strenuous exercise challenges this control, because it is markedly thermogenic, owing to its stimulation of metabolic rate. Recall that the energy-producing systems are <40% efficient, and the remainder of the energy is given off as heat. The fact that, on average, three heat-related deaths have occurred in football each year since 1995 attests to the seriousness of hyperthermia.

Various mechanisms of thermoregulation maintain thermal balance in the body. Muscular activity is one of the most influential factors contributing to heat gain in body core temperature. Others include hormonal effects, the thermic effect of food, postural changes, and environmental changes. Countering the heat gain factors are mechanisms that protect against hyperthermia by removing heat from the body, including radiation, conduction, convection, and evaporation. In an otherwise normal person engaged in strenuous exercise, evaporation (of sweat) provides the most important physiological defense against overheating. Evaporation of 1.0 mL of sweat is equivalent to about 0.6 kcal of body heat loss. Therefore, even at maximal exercise—at which 4.0 L O₂/minute are consumed, equivalent to about 20 kcal/minute of heat produced—core temperature would be expected to rise just 1°F every 5 to 7 minutes. This gradual rise occurs because sweating, assumed to be maximal at 30 mL/minute, would cool the body only to the extent of about 18 kcal/minute.

Approximately 80% of the energy released during exercise is in the form of heat. If this heat is not removed from the body, the heat load from metabolic activity, combined with environmental heat during strenuous exercise, could lead to a dramatic increase in body temperature. Hyperthermia can result in lethal heat injury. You have read that the major mechanism for heat loss is the evaporation of sweat and that nearly 600 kcal are eliminated by the cooling effect of the evaporation of 1 L of sweat. Among the remaining mechanisms for heat removal, radiation is the next most important. In radiation, heat generated in the

working muscles is transported by blood flow to the skin, from which it can subsequently be exchanged with the environment. For both of these thermoregulatory mechanisms, body water is clearly the major participant, and therefore interest in assessing various strategies for replacing it during strenuous exercise has been considerable.

Firm evidence indicates that depletion of body water from sweating more than 2% of body weight can impair endurance considerably through deficiencies in thermoregulatory and circulatory functions. The most likely explanations for this impairment are as follows:

- A reduced plasma volume and therefore reduced hemodynamic capacity to achieve maximal cardiac output and peripheral circulation. As plasma volume declines, reduced skin blood flow and a fall in stroke volume follow. Heart rate increases to compensate but cannot offset the stroke volume deficit [1].
- Altered sweat gland function, whereby sweating ceases in an autonomic control attempt to conserve body water.

As a result of these reactions, body temperature rises quickly, drastically increasing the chance of cramps, exhaustion, and even heatstroke, a condition that has a mortality rate of 80%. Sweat losses of 1.5 L/hour are commonly encountered in endurance sports, and under particularly hot conditions sweat rates exceeding 2.5 L/hour have been measured in fit individuals. Marathon runners can lose 6% to 8% of body weight in water during the 26.2-mile event, and plasma volume may fall 13% to 18%. It is common, therefore, for a 150 lb runner to lose 0.5 lb of water (equivalent to an 8 oz glass of water) per mile in a hot environment.

Dehydration results when fluid loss exceeds intake, and the degree of dehydration is directly proportional to the fluid disparity. The primary goal of fluid replacement is to maintain plasma volume so that circulation and sweating can proceed at maximal levels. The maximum rate of sweating is greater than the maximum rate for absorbing water from the intestinal tract, so at maximum exercise effort, some dehydration is bound to occur. The endurance athlete has difficulty avoiding a negative water balance, because attempting to replenish the copious amount lost in the course of a marathon is both impractical and distasteful. It is distasteful because the necessary intake far exceeds the thirst desire, a stimulus that is delayed behind rapid dehydration. Athletes, when left to follow their thirst desire, replace only about half the water lost during

exercise [2]. Force-feeding of fluids to exactly balance fluids lost is ideal from the standpoint of athletic performance, although the dramatic effects of lesser amounts of fluid replenishment during exercise are also well documented. The experimental design on which such conclusions are generally based is comparison of the extent of fluid intake with performance and certain physiological parameters, such as heart rate and body temperature. Study groups are commonly composed of subjects who, in the course of prolonged exercise, are (1) force-fed fluids beyond the thirst desire, (2) allowed to drink fluid ad libitum (as desired), or (3) deprived of fluid intake. Force-fed subjects display superior performance, lower heart rate, and lower body core temperature than the other groups, and the ad libitum group outperforms the deprived group in these parameters.

An imposing question in sports nutrition that is surrounded by controversy is whether electrolyte replacement is necessary during prolonged exercise. Based on the knowledge that sweat contains electrolytes (sodium, potassium, chloride, and magnesium), it was reasoned that because substantial amounts of electrolytes were lost during endurance athletics, replacing them was necessary to optimize performance. In the 1970s, sports drinks supplemented with electrolytes and sometimes glucose (GES drinks) began to appear on the market and are currently sold under names such as Gatorade, POWERADE, and All-Sport. Whether such supplementation is necessary depends on the length and level of intensity of the exercise and therefore on the quantity of sweat lost. GES drinks containing about 8% or less of carbohydrate have been shown not to decrease stomach emptying time and provide rapid absorption. The nature of the carbohydrate is important. Polyglucose increases the amount of glucose absorbed and has a small effect on osmolality. The carbohydrates used include glucose, sucrose, fructose, high-fructose corn syrup, and maltodextrins, among others.

The electrolyte content of sweat in the average person is very low compared with body fluids. Table 1 compares the electrolyte concentrations of sweat and blood serum. In the case of sodium and chloride ions, dehydration through sweating has the effect of concentrating these electrolytes in extracellular and intracellular water because of their relatively low concentration in sweat. Therefore, in marathon-level exertion, in which a total sweat loss of 5 to 6 L or less is incurred, rehydration with water alone is adequate, because only about 200 mEq of sodium and chloride would be lost from a relatively large body store.

Table 1 Average Electrolyte Concentrations in Sweat and Blood Serum (mEq/L)

	Na ⁺	K ⁺	Cl ⁻	Mg ²⁺
Sweat	40–45	3.9	39	3.3
Blood serum	140	4.0	110	1.5–2.1

Some research suggests that good-tasting electrolyte-glucose solutions encourage the athlete to consume more fluid than would be consumed using only water as a replacement fluid. The enhanced fluid intake therefore is probably beneficial, even though the electrolytes might not be needed. Drinking water would have the effect of lowering the osmolality of the blood, which would reduce the thirst reflex.

The American Academy of Pediatrics makes recommendations for exercising children, including the use of flavored beverages that contain electrolytes, for the reasons stated above [3]. Although some researchers regard potassium losses during exercise in the heat as constituting a potential health problem, this view too is controversial given the relatively small amount of the ion lost. On the basis of the potassium content of sweat (shown in Table 1), a sweat loss of 5 L would induce an

estimated potassium deficit of <20 mEq, or well under 1% of the estimated total body store of 3,000 mEq for a 70 kg man.

Only under severe conditions of prolonged, high-intensity exercise in the heat would electrolyte replacement be indicated. In such a case, electrolyte loss may exceed the amount provided in the daily diet, and some sodium supplementation may be necessary. The quantity provided by adding one-third teaspoon of table salt to a liter of water would be adequate [4]. Whether potassium supplementation is called for under similar conditions is doubtful, for the reasons discussed.

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www.umass.edu/cnshp/index.html

Center for Nutrition in Sport and Human Performance at the University of Massachusetts.

www.gssiweb.org

A web site of the Gatorade Sports Science Institute. It contains many summary articles on exercise science, sports nutrition, and sports medicine.

www.beverageinstitute.org

Web site sponsored by the Coca-Cola Company and POWERADE. It contains articles at the consumer and professional level on fluid and beverage intake.

Experimental Design and Critical Interpretation of Research

The Scientific Method

Historical Applications

Research Methodologies

Historical Method (Qualitative)

Descriptive Survey Method (Qualitative)

Analytical Survey Method (Quantitative)

Experimental Method (Quantitative)

Randomized Clinical Trial

Terms That Describe Research Quality

Initiation of Research

Problems and Pitfalls in Research

Evaluation of Research and Scientific Literature

Nutrition Research on the Internet

Research is a process that seeks, finds, and transfers new knowledge. Although many definitions of research have emerged, probably none is more comprehensive and poignant than that taken from *Webster's Dictionary of the English Language*:

Research: A studious inquiry or examination, especially a critical and exhaustive investigation or experimentation having for its aim the discovery of new facts and their correct interpretation, the revision of accepted conclusions, theories, or laws in the light of newly discovered facts or the practical application of such conclusions, theories, or laws.

At the core of this definition is that research discovers new facts (if there is no discovery, there was no research) and then correctly interprets those facts.

All of the information in this text was derived from research, and the reference list at the end of each chapter enables the reader to examine the source of the information. Publications cited in bibliographies of the chapters reveal to the reader the premise or justification of the research, the experimental method by which it was conducted, and the author's interpretation of the results. Through such publications, findings and facts in the nutrition arena are routed through the appropriate scientific journals to the public. Perhaps more than any other discipline, nutrition furnishes an unrelenting barrage of news through the media, because nutrition touches the lives of all of us by appealing directly to our concept of good health. Unfortunately, along with the profusion of nutrition news comes the probing question as to what is reliable and what is not. It is imperative that the student of nutrition recognize this problem and learn to separate fact (derived from carefully constructed research) from fictional information passed along through anecdotal reports and hearsay.

The refereed or peer-reviewed journals offer the best source of information. The research procedures and results submitted by the investigator to these journals for publication are critically examined by other scientists knowledgeable in that particular area of research. This peer review process screens proposed publications for quality and soundness, ensuring the reliability of research papers that are ultimately published in that journal. Journals that feature nutrition-related investigations that have won the respect of practitioners over the years include *The American Journal of Clinical Nutrition*, *Nutrition Journal*, *Nutrition Reviews*, and *Journal of the American Dietetic Association*. Many other excellent journals frequently publish nutrition-related reports. Students who regularly consult the nutrition literature will learn to recognize these and distinguish them from weaker publications. The qualifications of the suppliers of information in other than peer-reviewed journals should be examined carefully and the reliability of the information judged accordingly.

As its title indicates, this chapter seeks to acquaint the reader with the various experimental methodologies (designs) available to the researcher and to offer

information that may be helpful in both understanding research terminology and critiquing existing research publications.

The Scientific Method

Research uses a process called the scientific method to solve problems or to resolve previously unanswered questions. The scientific method contains the following fundamental components:

1. *The research purpose or problem*: expresses the question to be answered or the problem to be solved.
2. *The hypothesis*: predicts the outcome of the research that will follow and therefore a solution to the problem or an answer to the question.
3. *Experimentation*: describes how the research itself was conducted, using one of the many methods available to the researcher.
4. *Interpretation or analysis*: interprets the data collected from the experimentation so as to understand what it means.
5. *Conclusion*: answers the originally posed question, and confirms or disproves the hypothesis.
6. *Theory formulation*: presents a statement founded on the conclusion.

Research can be thought of as occurring in a cyclic process, with the components of the scientific method arranged as illustrated in Figure 15.1. However, thinking of research in terms of a closed circle may be misleading. Research does not dead-end into finality with the theory statement. To the inquisitive mind of the researcher, a conclusion from one experiment invariably gives rise to new questions and problems. Research is therefore non-ending, and its cyclic nature may be more meaningfully represented as a spiral or helix, twisting inexorably upward toward a limitless goal.

Research design, particularly that which lends itself to the physical and biological sciences, must include the components listed. To be considered a fact, the theory

formulated must be verified by other investigators who carry out the experiment under the same controlled conditions used in the original research. If the theory is verified, it becomes accepted as fact until further advances in research can disprove it.

HISTORICAL APPLICATIONS

Antoine Lavoisier is credited with being the first to implement the scientific method in his research, which he conducted during the 18th century. Up until that time, inquiry into problems had been only a philosophical exercise. His approach to problem solving is illustrated by the following steps [1,2]:

- Using Priestley's earlier discovery that oxygen is involved in burning, Lavoisier formulated the hypothesis that respiration in animals was a form of combustion.
- Lavoisier carried out experimentation under controlled conditions on animals (guinea pigs). Oxygen consumption, heat production, and production of carbon dioxide by animals confined in airtight chambers were carefully measured.
- From the measurements (data) collected, Lavoisier interpreted the results: a pattern could be identified between oxygen consumption, carbon dioxide production, and heat emanating from the animal body.
- Based on his interpretation of the data, Lavoisier formulated the theory that consumption of oxygen is related to the amount of carbon burned or heat produced in the animal body. Then, to validate his theory, he performed similar controlled experiments on other animals, including humans.

Nutrition research, designed to expand the nutrition knowledge base, began with Lavoisier's revelations about energy metabolism (around 1789) [2] and moved at a rather slow pace until the beginning of the 20th century. At about that time, many important discoveries and technological advances enabled a rapid expansion of the nutrition knowledge base. Nutrition research continued to flourish throughout the 20th century.

Research Methodologies

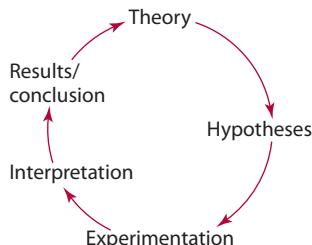


Figure 15.1 The cyclic nature of research from theory to conclusion.

Many different types, or classifications, of research exist. One of the broadest classifications of research is according to *application*: is the research basic or applied? Basic research seeks to expand existing knowledge by discovering new knowledge. Applied research, in contrast, seeks to solve problems primarily in a field setting. Research may also be classified according to *strategy* (historical, survey), *degree of experimental control* (experimental vs. nonexperimental),

time dimension (cross-sectional vs. longitudinal), *setting* (laboratory, field), or *purpose* (descriptive or analytical).

Despite the diversity of research classifications, the methods by which research can be carried out are more concisely categorized. Two broad methodological approaches encompass essentially all research: *qualitative methods* and *quantitative methods*. They are distinguished from each other according to the nature of the data collected in the study. All the data emerging from a research study reaches the researcher in the form of either words or numbers. *If the data are verbal, the method is qualitative; if the data are expressed in numbers, the method is quantitative.* Within these major categories, four discrete subdivisions of methodological approaches can be used in research: historical methods and descriptive survey methods, both of which are qualitative approaches, and analytical survey methods and experimental methods, which are quantitative in nature. This chapter describes each of these methods. The text by Leedy [3] provides a good review of research methodologies.

HISTORICAL METHOD (QUALITATIVE)

Historical research seeks to explain the cause of past events and to interpret current happenings on the basis of these findings. Sources of information for the historical researcher are primarily documentary, existing in the form of written records and accounts of past events as well as literary productions and critical writings. The researcher relies, if possible, only on primary data, that is, data that are "firsthand" and therefore minimally distorted by the channels of communication. Generally, information gathered by historical research does not need to be analyzed by any form of statistical treatment or data analysis.

DESCRIPTIVE SURVEY METHOD (QUALITATIVE)

A word that distinguishes the descriptive survey method from other research methods is *observation*. The investigator observes, across a defined population group, whatever variable is under study. The variable may be physical (size, shape, color, strength, etc.) or cognitive (achievement, beliefs, attitudes, intelligence). The researcher first observes very closely the population bounded by the parameters that were set for the study and then carefully records what was observed for future interpretation. Note that observation involves not only visual perception but also (most likely) tests, questionnaires, attitude scales, inventories, and other evaluative measures. In fact, most descriptive surveys use well-designed questionnaires as the instrument of observation.

Keeping records, which can be thought of as a way of preserving facts, is an important feature of the descriptive survey method. Observation and record keeping are exemplified by the case study, which follows the symptomatology, treatment, conclusions, and recommendations

as they apply to a patient under study. A case report is a report of observations on one subject, whereas a case series involves observations on more than one subject. Generally, the subjects being observed have a condition or disease in common. This form of research design is useful in attempts to identify variables or generate hypotheses that may be important in the etiology, care, or outcome among patients with a particular disease or condition.

ANALYTICAL SURVEY METHOD (QUANTITATIVE)

The analytical survey method is best described by contrasting it with the descriptive survey method just outlined. While the descriptive survey method involves observations that can be described in words and conclusions drawn from those words, the analytical survey uses a language not of words but of numbers. Because values obtained from an analytical survey are numerical, the data are said to be quantitative.

The quantitative data of an analytical survey are analyzed by statistical tools to produce numerical results from which conclusions can be inferred. Statistical analysis of numerical data may include:

- measures of central tendency (mean, median, mode)
- measures of dispersion (range, standard deviation, coefficient of variation)
- measures of correlation (correlation coefficient, regression analysis)

These measurements fall within a category called descriptive statistics. Another category of statistics, called inferential statistics, has two principal functions:

- to predict or estimate from a random sample a certain parameter in a general population
- to test null hypotheses based on statistics

The null hypothesis postulates that no statistically significant difference exists between phenomena that occur by pure chance and the statistically evaluated behavior of the data as they have been observed by the researcher. As an illustration of testing the null hypothesis, suppose a study is to be conducted on the level of serum iron among vegetarians and nonvegetarian, omnivorous consumers. In keeping with a quantitative study, the data will be numerical; in this case, serum iron concentration expressed in milligrams per deciliter. Before the data are collected, the null hypothesis holds that there is no statistical difference between the two groups in the study criterion (serum iron concentration). If, in fact, the experimental statistical findings confirm this prediction, the null hypothesis is said to be accepted. However, if the findings show that a statistically significant difference exists between the two groups in the level of serum iron, then the null hypothesis is rejected.

Observational research designs can also be included in the category of analytical surveys. The use of the word *observational* in describing this type of research may at first seem confusing, because observation is also the hallmark of descriptive surveys. Remember, however, that the observational research designs described here are examples of an analytical study and, as such, produce numerical, quantitative data that can be interpreted by applying statistics. Descriptive surveys, on the other hand, though based on observation, rely on written data and are qualitative, not quantitative.

Observational designs may take the form of epidemiological or cohort studies, neither of which involves experimentally induced changes in variables. **Epidemiology** has been defined as “the study of the distribution of a disease or condition in the population, and the factors that influence the distribution.” A **cohort** is a group of subjects entered into a study at the same time and followed up at intervals over a period of time. A cohort study is also called a prospective (“looking ahead”) study.

The results and conclusions of an experiment, and eventually the establishment of scientific fact, ultimately depend on the statistical treatment of data, a field of considerable scope. This section is intended to simply acquaint the reader with some statistical terms commonly used in the analysis of numerical data. For the interested reader, many comprehensive texts on applied statistics offer a more in-depth treatment of the subject [4,5].

EXPERIMENTAL METHOD (QUANTITATIVE)

Among the research methodologies, the experimental method is the one most commonly encountered in the nutrition literature. The hallmark of the experimental method is control. So basic is control to this method that it is frequently called the control group–experimental group design. Such a study uses two or more population groups with the subjects of each group matched, characteristic by characteristic, as closely as possible to the subjects of the other group(s). One group serves as a control and as such is not exposed to any extraneous change. The experimental group is exposed to the alteration under study, and whatever change is noted in this group relative to the subjects of the control group is presumed to be caused by the extraneous variable(s).

The experimental method can also use just one group, a method sometimes called a pretest–post-test approach. In its simplest form, a group of subjects is first evaluated (pre-test) and then subjected to the experimental variable (test) and reevaluated (post-test). So-called crossover studies are also commonly used. In this type of study, a control group is not subjected to a particular experimental variable, while an experimental group is subjected to the variable, and differences in the data are noted. Then the original control group is exposed to the variable, therefore becoming

the experimental group, and the original experimental group is not exposed to the variable and thus becomes the control group. This approach corrects for any inherent differences in the two groups that might confound the experimental data.

In short, the experimental method is based on cause and effect. It involves intervention on the part of the researcher, who introduces a variable and records its effect. Experimental research designs enable the investigator to control or manipulate one or more variables in an effort to examine the relationship between the variables. Variables typically are designated as dependent and independent. The independent variable is the variable controlled or manipulated by the investigator. The dependent variable occurs as the result of the influence of the independent variable. In other words, the dependent variable reflects the effects of the independent variable. As in the case of the analytical survey method, many traditional descriptive and inferential statistical tools can be used to analyze the data.

Because the experimental method is so commonly used in nutrition research, numerous examples could be given to illustrate its use. A classic illustrative example of research that employs the experimental method is the randomized clinical trial discussed in the next section.

RANDOMIZED CLINICAL TRIAL

The randomized clinical trial, an experimental research design, is frequently used in medical research studies involving humans. Randomized clinical trials are usually conducted after preliminary trials have been done with experimental animals, and they typically test the benefits of one or more treatments. Subjects in randomized clinical trials are those who have the condition to be treated, and they should be representative of the population to which results are to be applied. Subjects are assigned randomly to a treatment group. In some instances, only one treatment is available, and a placebo is used for the control group. Subjects who enroll in the study must be informed that they have an equal chance of being assigned either to the treatment group or to the control (placebo) group. Ideally, to avoid bias, a clinical trial should be “double blind,” with neither the subject nor the investigator knowing which group is which.

Terms That Describe Research Quality

Descriptive terms that reflect the effectiveness or quality of a research effort include:

- validity
- accuracy

- reliability
- precision

The “truth” of research lies within the validity of its collected data. Validity represents the extent to which the process or technique being used is measuring what it is supposed to be measuring. *Validity is concerned with the effectiveness of the measuring instrument.* The term *instrument*, as it applies in research, is broadly defined, with applications ranging from survey questionnaires to pieces of scientific equipment.

Several types of validity exist. Face validity relies on the subjective judgment of the researcher and involves asking the following questions: (1) Is the instrument measuring what it is supposed to be measuring? (2) Does the sample being measured adequately represent the behavior or trait being measured? Criterion validity uses as an essential component a reliable and valid criterion (i.e., a standard against which to measure the results of the instrument that is doing the measuring). Validity can also be expressed as internal or external, and both types are very important in research. Internal validity refers to causal relationships, that is, whether an experimental treatment made (caused) a difference. External validity refers to the generalizability of the results of the research to a population group that was not studied.

The terms *accuracy* and *reliability* are related, because both are concerned with how close to the “truth” a measurement is. Accuracy is expressed as the difference between the measured values of an instrument and the true values. The more accurate a measurement, the closer the result is to the true value. Reliability refers to the instrument used in the study and indicates the degree of accuracy that it generates. An instrument may be reliable within a broad range of accuracy. This concept can be illustrated by the use of a sundial as an instrument for telling time. It is a reliable timepiece if one is concerned only with whether the time of day is early afternoon or late afternoon. However, the sundial has poor reliability for more specific timing, such as informing the observer when it is time to turn on the television to see a favorite show or to leave to catch a bus. In both of these cases, the sundial’s accuracy, that is, the quantitatively expressed nearness of the measured time to the true time, is poor.

The term *precision* is a very useful expression of the consistency or repeatability of multiple analyses performed on the same sample or subject. Procedures used in research, when they are repeated, should generate the same data from the same sample. For example, multiple assays of serum glucose performed on the same serum sample provide an indication of the precision of the instrument used. Understanding the difference between precision and accuracy is important. A method may be highly precise (i.e., replicate values may be very close to one another) yet not be accurate. In contrast, widely disparate replicate values

(i.e., poor precision) might yield an accurate average value. However, imprecise measurements are not a property of quality research.

Initiation of Research

The only prerequisite for initiating research is an inquiring mind. The novice is likely to be intimidated by reports of sophisticated research that has required expensive equipment, extensive personnel, and a generous budget. Not all research needs to be conducted at this level. It can be simple and inexpensive while still serving the purpose of broadening the knowledge base.

Initiating research requires familiarity with the characteristics of research. These characteristics are shown in Table 15.1. The first characteristic suggests that research begins with a question: Why does something occur, or what causes something? Research demands that the problem be identified and stated clearly. Research requires a plan. It seeks direction through hypotheses. Research deals with data and their meaning. And, as shown in Figure 15.1, research is circular. To be certain that all these characteristics are included in a research project, the following four steps should be followed [6]:

1. *Select the research topic or problem to be solved.* Choosing a topic or problem narrow enough to be manageable often can be difficult. A review of published literature

Table 15.1 Checklist for the Evaluation of Research

- | |
|---|
| Is the central problem for research (and its subproblems) clearly stated? |
| Does the researcher provide evidence for a plan and organization? |
| Has the researcher stated his or her hypotheses? |
| Are the hypotheses related to the principal problem or the subproblems of the research? |
| Are the assumptions stated? Are these assumptions realistic for the research undertaken? |
| Is the researcher methodology that has been employed clearly stated? |
| If the research is for experimental design, answer the following questions: |
| Is the study <i>in vitro</i> or <i>in vivo</i> ? |
| Does the study use humans or experimental animals? |
| What are the age, sex, and number of subjects in each experimental group? |
| What is the length of time of the experiment? |
| Is there sufficient statistical power in the design (number of subjects, length of time of the experiment, and size of the anticipated change)? |
| Is the statistical treatment of data clearly defined, and are statistics presented in a straightforward manner? |
| Are the conclusions that the researcher presents justified by the facts presented? |
| Is there any indication whether the hypotheses are supported or rejected? |
| Are limitations of the study identified? |
| Is there any reference to or discussion of related literature or studies by other investigators? |
| Are specific areas for further research suggested? |
| By whom was the research sponsored? Could results be influenced in any way by the source of funding? |

that is related to the selected research topic is necessary to provide a basis on which to build present research and precisely define the research.

2. *Clearly state the question to be researched.* Components of the question include who or which (i.e., the subjects or units being assessed are identified), what (i.e., the factor of interest is stated specifically), and how assessed (i.e., the outcome to be assessed is stated specifically).
3. *Prepare a research plan or proposal.* The proposal should include several elements:
 - a statement of the research question (from step 2)
 - a review of literature (from step 1)
 - an explanation of the scientific and/or social relevance (importance) of the research
 - a description of research design, which should specify the specifics of the investigation (i.e., methods, data analysis, and the appropriate statistical analysis)

Putting the plan in writing forces the researcher to think through all aspects of the investigation and can serve as a clearly defined guide for carrying out the project. The plan becomes a working document that can be converted into the research report. A written plan is more likely to be followed without modification over time.

Depending on the level of the research, the plan may range from a simple outline to a complicated, detailed request for funding from a foundation or government agency. Regardless of the level of investigation, established guidelines must be followed whenever live (human or experimental animal) subjects are used. Review of proposed research projects by committees on ethical standards ensures that procedures are acceptable. These committees operate in academic institutions at the departmental and university level. Funding agencies and organizations are very careful about considering only those proposals that strictly adhere to the guidelines.

4. *Plan for collecting and preparing data.* Once the method for data collection has been selected (or designed), a pilot study can be conducted to point out any adjustments or modifications that need to be made. The pilot study also may provide a good indication of the value of the data being collected. Improvements in research design often result from a pilot study.

Once the research procedures have been refined, the planning stage is finished, and the research study can be conducted. Carrying out the research involves collecting data, followed by the crucial steps of interpreting the data and reporting the results. The problem that initiated the research finally is addressed, and results of the investigation are interpreted in the framework of existing theory and past research, if any is available. Either the problem is solved, or the process must begin again. Many valuable

outcomes are possible from even the simplest research projects if they are well planned. An interest in problem solving coupled with diligence in planning an orderly, stepwise progression in problem solution provides the essentials for scientific inquiry.

Problems and Pitfalls in Research

In general, a clear understanding of the steps or components of the research process provides a good checklist against which to evaluate research presented in publications or to plan one's own research study. Attention to these components also provides a guide to problems and pitfalls that can plague research.

The logical progression of components in the research process was described in the previous section and reproduced in checklist form in Table 15.1. A complete plan for this logical progression of the elements of research (including the question, research design, and exact statistical analysis) should be in place before any research activity begins. If this plan is completed, then the research study will in fact be following a predetermined protocol, in a manner similar to a National Aeronautics and Space Administration (NASA) launch of a manned space probe.

Despite the best-laid plans, some problems can occur during a research study. As summarized in an editorial by Vaisrub [7], problems that can be considered as either "soluble" or "insoluble" may arise in the course of a research study. Examples of both types of problems are given in Table 15.2.

Table 15.2 Commonly Encountered Problems in Research

Insoluble problems in studies:

- Lack of representative sampling
- Vague target population definition with poor selection or subjects
- Lack of random allocation of treatments
- Lack of proper handling of confounding (nuisance) variables
- Lack of appropriate controls
- Lack of blinded subjects and evaluators
- Lack of objective measurements or assessment of outcome

Possibly soluble problems in studies:

- Inadequate assurance of group comparability
- Inappropriate choice of sample units
- Use of calculated normal limits for skewed distributions; multiple significance testing
- Incorrect denominators for rates, risks, or probabilities
- Misuse and incorrect presentation of age data
- Improper handling of problems arising from incomplete follow-up in longitudinal studies
- Spurious associations between diseases or between a disease and apparent risk factors
- Ambiguity concerning descriptive statistics used

One of the more difficult and error-prone areas of research is in applying statistics to analyze the data. Typically, this process involves rejecting or retaining a null hypothesis. If the statistics are invalid, the null hypothesis may be rejected when it should have been retained, or it may be retained when it should have been rejected. The most common cause of such errors is insufficient power of the statistical test (“power” refers to the likelihood of falsely rejecting a null hypothesis). In view of the magnitude of the subject, statistical data analysis is not addressed in this chapter. However, excellent pertinent references are available to the interested reader [3,4,8].

Evaluation of Research and Scientific Literature

Although the library “research” paper cannot be considered research because it is not gathering new data or using existing data for a new purpose, it does involve selecting and transferring existing information and therefore requires careful evaluation of scientific literature. Like initiating research, evaluation requires familiarity with the characteristics of research. The questions posed in Table 15.1 can serve as a guide in identifying the quality of published research articles. The type of publication in which the research article is published is also important. Publication in a peer-reviewed or refereed journal indicates that the article has been reviewed by some of the researcher’s peers to determine its worth for publication. Although peer review helps enhance the quality of a research publication, it is not a guarantee of a high-quality study.

One form of research synthesis attempts to solve research problems through literature reviews. A popular technique to accomplish this goal is called meta-analysis. A full discussion of the technique is beyond the scope of this text, but briefly, it is a way to quantitatively compare (using statistical measures) treatment effects from studies with similar treatments. Meta-analysis involves additional steps beyond the typical literature research review paper. After the problem to be studied is identified, definitive criteria are reported for the literature analysis. Additionally, the results included from the various studies are converted to a quantitative standard that enables statistical techniques to be used as a means of analysis [9].

Although most quality research articles appear in refereed journals, many excellent invited reviews from prestigious investigators may appear in other journal publications, such as *Nutrition Today*, *Nutrition in Clinical Practice*, or *Contemporary Nutrition*. These reviews are not original research but are summaries of research in a particular area (subject) and are based on information formerly published in refereed journals. What distinguished these reviews from

meta-analysis is the lack of a quantitative standard and statistical analysis of the data from multiple published reports. The information in review articles is secondhand and thus may have become somewhat distorted because of imperfections inherent in communication. Review articles, however, can be extremely helpful in providing an overview of some particular topic. When specifics are important, the original report should always be consulted.

The introduction to this chapter emphasizes that because of intense public interest in nutrition and health, the media floods us with nutrition-related information. This information wears two faces: that based on sound scientific research and that which comes from anecdotal reporting, hearsay, or quackery. The well-informed student of nutrition must learn to distinguish these and to critically evaluate the source of a report. This distinction may be easy to make at the extremes of “good” science and quackery, but many times information falls between these extremes, and distinguishing between the two becomes more of a challenge. Articles designed to help the nutrition student make such distinctions appear frequently in respected literature sources. Two such articles are referenced here [10,11].

Nutrition Research on the Internet

Nutrition is big business on the Internet, just as it is in all other forms of mass communication. Nutrition topics take many popular guises: weight loss diets and healthy heart diets, recipes for lowering cholesterol, tips on how to reduce the risk of cancer or boost sports prowess, and herbal therapies for all of these purposes.

Professional nutrition research on the Internet should follow all the guidelines of good research described in this chapter. In addition, users of the medium must recognize some Internet-specific caveats. In seeking out new information on the Internet while at the same time attempting to separate hearsay, anecdotal reporting, and quackery from authentic information, the reader should ask the following questions:

- **What is the source of the web site?** Most web sites have owners, sponsors, or both who may have a proprietary interest in promoting a product or an agenda. Also, because the web is worldwide, some web sites originating in another country may use different guidelines or principles. The source is always posted, sometimes in small print at the bottom of the home page.
- **Who are the contributors?** Nutritionists and health care professionals should be prominently listed as contributors to a web site. For example, the web site medicinenet.com has a page listing the contributing members of its medical advisory board.

- *Is the web site efficiently managed?* The site should be frequently updated. Generally, the most recent updates are posted. Within the limits of the computer used, moving between pages should be quick and easy. A “search” component, to access all the resource information of the web site, should also be available.
- *What links to other web sites and databases are provided?* The site should provide links to other reputable professional sources of information such as the National Library of Medicine’s MEDLINE/PubMed, which holds records and abstracts from over 3,500

SUMMARY

This chapter identifies characteristics of research, notes the process for evaluating scientific literature, and identifies problems that can plague a research study. It also describes methodologies used in research.

Certainly, one chapter cannot provide sufficient depth of information for performing outstanding research. Study and coursework in the various elements of the research process, as well as apprenticeship to a more experienced investigator,

medical journals and other publications. Databases that are accessed should provide abstracts of the research publications. In some cases, complete articles can be ordered online.

Research on the Internet is novel, convenient, and appealing, but remember that it is simply a technologically advanced medium designed to disseminate vast amounts of information to, in many cases, unwary users. The information it provides must be evaluated for authenticity at least to the same degree as information accessed through the more traditional library search.

are normally required. We hope, however, that the material offered in the chapter, together with the supplemental references, will at least arm the reader with new insight into proper research protocol and impart a higher level of confidence in becoming a critical reviewer of the literature.

Expanding the nutrition knowledge base depends on ongoing nutrition research at every level. Knowledge about the total human depends on research at the molecular, cellular, organ or tissue, and system levels.

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Suggested Readings

Monsen ER, Cheney CL. Research methods in nutrition and dietetics: Design, data analysis and presentation. *J Am Diet Assoc* 1988; 88:1047–65.

This extremely useful article can serve as a guide for students who are learning the mechanics of research as well as for practitioners in the field of nutrition. Practical examples of research that could be conducted inexpensively in the work setting are given.

The Surgeon General's Report on Nutrition and Health. DHHS (PHS) publication no. 88-50210. Washington, DC: U.S. Government Printing Office, 1988.

This 725-page report includes extensive evidence for the relationship between nutrition and several chronic diseases. Results of

research reported to support evidence of relationship are based on a wide array of studies: dietary studies, experiments with laboratory animals, genetic and metabolic research, and epidemiological studies. Issues of special priority for continuing research are listed after discussion of each disease.

Touliatos J, Compton N. *Research Methods in Human Ecology and Home Economics*. Ames, IA: Iowa State University Press, 1988.

This is an excellent up-to-date book on the how-to's of applied research.

Web Sites

- www.eurekalert.org
EurekAlert; American Association for the Advancement of Science
- www.cspinet.org
Center for Science in the Public Interest
- www.quackwatch.com
Quackwatch, member of the Consumer Federation of America
- www.nlm.nih.gov
National Library of Medicine: MEDLINE/PubMed
- www.medicinenet.com
A consumer-oriented health web site.
- www.nejm.org
New England Journal of Medicine
- www.ama-assn.org
Journal of the American Medical Association
- www.ncahf.org
National Council against Health Fraud
- www.ilsi.org
Nutrition Reviews
- www.ajaxn.org
American Journal of Clinical Nutrition

Glossary

Achlorhydria Lack of hydrochloric acid in gastric juice.

Activation energy Energy introduced into the reactant molecules to activate them to the transition state so that an exothermic reaction can take place.

Acute Having a rapid or sudden onset.

Alkalosis A condition in which the pH of the blood is above 7.45.

Amenorrhea The absence of at least three consecutive menstrual cycles.

Amphibolic pathway A pathway that is involved in both the catabolism and the biosynthesis of carbohydrates, fatty acids, and amino acids.

Amphipathic Refers to a molecule that has a polar region at one location and a nonpolar region at another.

Amphoteric Something that can react as either an acid or a base.

Anomeric carbon The carbon that forms a ring structure with the reducing carbon reacting with the OH group on the highest numbered chiral carbon of a monosaccharide.

Anticodons Three-base sequences of nucleotides on molecules of a transfer RNA (tRNA).

Antral Pertaining to the antrum, the lower or distal portion of the stomach.

Apolipoprotein The protein component of a lipoprotein.

Apoprotein A protein that binds to ligands (other compounds).

Apoptosis An organized series of events that, once triggered, leads to cell death.

Aromatic compound An organic compound that contains a benzene ring.

Ataxia Impaired muscle coordination, especially when trying to perform voluntary muscular movements.

Atheroma A mass of plaque consisting of degenerated, thickened arterial intima, occurring in atherosclerosis.

Autolysis The digestion of intracellular components (including organelles) by lysosomes.

Autophagy The breakdown or digestion of the body's proteins, such as those found in the blood or within cells.

Beriberi A condition resulting from a thiamin deficiency.

Bile A body fluid made in the liver and stored in the gallbladder that participates in emulsifying fat and forming micelles for fat absorption.

Buffer A compound that ameliorates a change in pH.

Calpains A calcium-dependent protease involved in protein turnover in the body.

Carboxylation The addition of a carboxyl group to a molecule.

Catabolism The process by which organic molecules are broken down to produce energy.

Cathepsins A group of enzymes involved in breaking down or digesting the body's proteins.

Cells The basic units for all organisms that arise from preexisting cells.

Chaperones Soluble intracellular proteins that bind to and deliver minerals to specific intracellular locations.

Chelators Small organic compounds that form a complex with another compound, such as a mineral.

Chemiosmotic theory A process by which protons move down an electrochemical gradient, and the energy generated is used to phosphorylate ADP to make ATP.

Chiral carbon Carbon atoms with four different atoms or groups covalently attached to them.

Chronic Long and drawn out in duration.

Chylomicron A type of lipoprotein that transports lipids and lipid-soluble vitamins from the intestine into the lymph and then the blood for use by body cells.

Chylomicron remnant The portion of a chylomicron that is left after blood lipoprotein lipase removes part of its triglycerides.

Chyme Partially digested food.

Cobalophilins A group of proteins, sometimes called R proteins, that are found in digestive juices and bind to vitamin B₁₂ to facilitate absorption.

Codon A three-base sequence in a DNA or mRNA molecule that specifies the location of a single amino acid in a polypeptide chain.

Cohort A group of individuals that share common characteristics.

Colloids Substances comprised of very small particles that are suspended uniformly in a medium.

Colorimetric titration A method of measuring the volume of one reagent required to react with a measured volume of another reagent, using an indicator that changes color.

Complementary base pairing The pairing of nucleotide bases in two strands of nucleic acids; A pairs with T or U, G pairs with C.

Complete protein A protein that contains all the essential (indispensable) amino acids in the approximate amounts needed by humans.

Connexin A protein involved in forming junctions between cells.

Coulometric titration A method for determining the amount of a substance released during electrolysis by measuring the electrical charge. (Note: A coulomb is a unit of electrical charge.)

Cytochromes Heme-containing proteins that serve as electron carriers in oxidative phosphorylation.

Cytokines A generic term for non-antibody protein messengers released from a macrophage or lymphocyte that is part of an intracellular immune response.

Cytoplasm The continuous aqueous solution of the cell and the organelles contained in it.

Cytoplast A cell from which the nucleus has been removed.

Cytoskeleton Microtubules and microfilaments in the cell that provide internal reinforcement and communication.

Deamination The removal of an amino (NH_2) group from an amino acid.

Dehydrogenases Enzymes that catalyze reactions in which hydrogens and electrons are removed from a reactant.

Desaturation The process of converting a saturated compound to an unsaturated one.

Dietary fiber Nondigestible (by human digestive enzymes) carbohydrates and lignin that are intact and intrinsic in plants.

Dipeptidylaminopeptidase A protein-digesting enzyme that breaks apart dipeptides.

Direct calorimetry A method of measuring the dissipation of heat from the body.

Disaccharides Sugars formed by combining two monosaccharides through a glycosidic bond between the hydroxyl group of one monosaccharide and the hydroxyl group of another.

Dowager's hump A deformity of the spine characterized by a humpback or being bent forward; also called *kyphosis*.

Eicosanoids Biologically active substances derived from arachidonic acid.

Electron transport chain The sequential transfer of electrons from reduced coenzymes to oxygen that is coupled with ATP formation.

Elongation The extension of the polypeptide chain of the protein product during protein synthesis.

Endocrine system All of the body's hormone-secreting glands.

Endocytosis Uptake of a substance into a cell through the formation of vesicles derived from the plasma membrane.

Endopeptidase An enzyme that hydrolyzes amino acids linked to other amino acids in the interior of a peptide or protein.

Endoplasmic reticulum (ER) A network of membranous channels pervading the cytoplasm and providing continuity between the nuclear envelope, the Golgi apparatus, and the plasma membrane.

Endothermic (reaction) A reaction in which the products have more free energy than the reactants; it therefore requires energy.

Enkephalins Peptides that bind to opioid receptors found in the brain and gastrointestinal tract.

Enterocyte An intestinal cell.

Enterohepatic circulation The movement of a substance, such as bile, from the liver to the intestine and then back to the liver.

Enzymes Protein catalysts that increase the rate of a chemical reaction in the body.

Epidemiology The science concerned with studying those factors that influence the frequency and distribution of disease.

Equivocal Uncertain or ambiguous.

Erythrocyte A red blood cell.

Estimated average requirement The amount of a nutrient thought to meet the nutrient requirements of 50% of healthy individuals in a specified age and gender group.

Eukaryotic cells Cells with a defined nucleus surrounded by a nuclear membrane.

Exocytosis A process by which compounds may be released from cells.

Exons The segments of a gene that code for a sequence of nucleotides in a specific molecule of mRNA.

Exopeptidase An enzyme that hydrolyzes amino acids off the terminal end of a peptide or protein.

Exothermic (reaction) A reaction in which the reactants have more free energy than the products; it therefore gives off energy as heat.

Exudate Fluids that have exuded (been forced or pressed) out of a tissue or its capillaries.

Ferment To break down substrates anaerobically to yield reduced products and energy.

Fermentation An anaerobic breakdown of carbohydrates and protein by bacteria.

Fibrotic Pertaining to fibrosis, formation of fibrous tissue as a reactive or repair process.

Free energy The potential energy inherent in the chemical bonds of nutrients.

Free radical An atom or molecule that has one or more unpaired electrons.

Functional fiber Nondigestible carbohydrates that have been isolated, extracted, or manufactured and have been shown to have beneficial physiological effects in humans.

Gap junctions Channels between cells.

Gene A section of chromosomal DNA that codes for a single protein.

Genome The sum of all the chromosomal genes of a cell.

Ghrelin A hormone secreted by the stomach and duodenum that signals hunger.

Gluconeogenesis The formation of glucose by the liver or kidney from noncarbohydrate precursors.

Glucose tolerance factor (GTF) A chromium-containing compound whose structure has yet to be characterized but may potentiate the action of insulin in the body.

Glycocalyx The layer of glycoprotein and polysaccharide that surrounds many cells.

Glycogenesis The pathway by which glucose is converted to glycogen.

Glycogenolysis The pathway by which glycogen is enzymatically broken down to glucose.

Glycolysis The pathway by which glucose is converted to pyruvate.

Glycoproteins Proteins covalently bound to a carbohydrate.

Glycosaminoglycan An unbranched polysaccharide consisting of alternate units of two different sugars.

Glycosidases/carbohydrases Digestive enzymes that hydrolyze polysaccharides to their constituent monosaccharide units.

Golgi apparatus The part of the cell responsible for modifying macromolecules synthesized in the endoplasmic reticulum and packaging them to be transported to the cell surface or cytoplasm.

Haptocorrins A group of proteins, sometimes called R proteins, that are found in digestive juices and bind to vitamin B_{12} to facilitate absorption.

Hartnup disease A hereditary disorder in which tryptophan absorption and excretion are abnormal.

Hemochromatosis An inherited disorder characterized by excessive iron absorption and iron overload in the body.

Heterodimers Complexes formed between two or more different receptors.

Hexosemonophosphate shunt The pathway that metabolizes glucose-6-phosphate to pentose phosphate, producing NADPH.

- Homeostasis** The tendency to stability in the internal environment of an organism.
- Homodimers** Complexes formed between two of the same receptors.
- Hormones** Chemical messengers synthesized and secreted by endocrine tissue (glands) and transported in the blood to target tissues or organs.
- Hydrolases** Enzymes that catalyze cleavage of bonds between carbon atoms and some other kind of atom by the addition of water.
- Hydroperoxyl radical** HO₂⁻ or H—O—O⁻.
- Hydroxyapatite** A crystal-lattice-like substance with the formula Ca₁₀(PO₄)₆(OH)₂, found in bones and teeth.
- Hypercalcuria** Excessive urinary calcium excretion.
- Hyperglycemia** A glucose blood level above normal.
- Hyperinsulinemia** A level of insulin in the blood that is above normal.
- Hyperkalemia** High concentrations of potassium in the blood.
- Hyperlipidemia** A general term for an elevated blood level of any lipid.
- Hyperphosphatemia** High concentrations of phosphorus in the blood.
- Hyperplasia** Excessive cell proliferation.
- Hyperpnea** An abnormal increase in the rate and depth of breathing.
- Hypertrophied** Grown larger or increased in size.
- Hypertrophy** Enlargement of the size of cells to increase the size of an organ.
- Hypocalcemia** Low concentrations of calcium in the blood.
- Hypochondriasis** Abnormal anxiety about one's own health.
- Hypoglycemia** A blood glucose level that is below normal.
- Hypokalemia** Low concentrations of potassium in the blood.
- Hyponatremia** Low concentrations of sodium in the blood.
- Immunoproteins** Proteins made by plasma cells that help destroy foreign substances in the body; also called *immunoglobulins* or *antibodies*.
- Indirect calorimetry** Measurement of the consumption of oxygen and the expiration of carbon dioxide by the body, used to estimate metabolic rate.
- Introns** Noncoding regions of a gene.
- In vitro** In a test tube or culture (outside the body).
- In vivo** Within the body.
- Ion** An electrically charged atom or group of atoms; positively charged ions are called *cations*, and negatively charged ions are called *anions*.
- Ischemia** Deficiency of blood in a tissue.
- Isomer** Two different chemical compounds that have the same molecular formula.
- Isomerase** Enzymes that catalyze the interconversion of optical or geometric isomers.
- Isoprenoid** Refers to the structure of the side chains of vitamins E and K.
- Isotope infusion** The direct introduction of an isotope (either radioactive or stable) into the bloodstream.
- Keratinocytes** Cells that produce the protein keratin.
- Ketone bodies** Compounds (acetacetate, β-hydroxybutyrate, and acetone) formed during the oxidation of fatty acids in the absence of adequate four-carbon intermediates.
- Krebs cycle** An aerobic metabolic cycle in the mitochondria that produces ATP; also called the *citric acid cycle*, or *tricarboxylic acid cycle*.
- Kyphosis** A deformity of the spine characterized by a humpback or being bent forward; also called *dowager's hump*.
- Lanugo** Fine, soft, lightly pigmented hair that usually is found on a fetus toward the end of pregnancy but may appear on malnourished individuals.
- Leptin** A polypeptide hormone secreted by adipose tissue that reduces hunger through hypothalamic mechanisms.
- Leukotrienes** Biologically active compounds derived from arachidonic acid.
- Ligands** Small molecules that bind to a larger molecule.
- Ligases** Enzymes that catalyze the formation of bonds between carbon and other atoms.
- Limiting amino acid** The amino acid with the lowest amino acid or chemical score; it is the amino acid present in a protein in the lowest amount, compared with a reference amount.
- Lingual** Pertaining to the tongue.
- Lipophilicity** The state of being attracted to lipids and thus repelled by water.
- Lipoproteins** Complexes of lipids and proteins that play a role in the transport and distribution of lipids.
- Lyases** Enzymes that catalyze cleavage of carbon-carbon, carbon-sulfur, and certain carbon-nitrogen bonds without hydrolysis or oxidation-reduction.
- Lysosomes** Cell organelles that contain digestive enzymes.
- Macronutrients** The dietary nutrients that supply energy, including fats, carbohydrates, and proteins.
- Marasmus** Malnutrition caused by prolonged intake of a diet deficient in energy (kcal).
- Metabolic syndrome** A clustering of a group of risk factors for cardiovascular disease, chronic kidney disease, and type 2 diabetes.
- Microflora** Bacteria adapted to living in a specific environment, such as the intestines.
- Microvilli** Extensions of intestinal epithelial cells designed to present a large surface area for absorbing dietary nutrients.
- Mitochondria** Cellular organelles that are the site of energy production by oxidative phosphorylation and the site of the tricarboxylic acid cycle; they are surrounded by an outer membrane that is very permeable and an inner membrane that is only selectively permeable.
- Monosaccharides** The simplest form of carbohydrates, which cannot be reduced in size to smaller carbohydrate units.
- Motility** Movement.
- Mucins** Glycoproteins found in some body secretions, such as saliva.
- Nervous system** The system of nervous tissue made up of neurons and glial cells.
- Nuclear envelope** A set of two membranes, which contain nuclear pores and surround the cell nucleus.
- Nucleoli** Regions of the nucleus containing condensed chromatin and sites for synthesizing ribosomal RNA.
- Nucleotides** A phosphate ester of the 5'-phosphate of a purine or pyrimidine in N-glycosidic linkage with ribose or deoxyribose, occurring in nucleic acids.
- Nystagmus** Constant, involuntary movement of the eyeball.
- Oligomer** Polypeptide chains joined to form a functional protein.
- Oligosaccharides** Short chains of monosaccharide units joined by covalent bonds.

- Oncogenes** Genes capable of causing a normal cell to convert to a cancerous cell.
- Ophthalmoplegia** Paralysis of the ocular muscles.
- oscilloscope** An instrument used to visualize echoes as part of an ultrasound examination.
- Osmosis** The net movement of the solvent (such as water) from a solution of lesser to one of greater concentration when the two solutions are separated by a membrane that selectively prevents passage of solute molecules but is permeable to the solvent.
- Osmotic pressure** A property of a solution that is proportional to the nondiffusible solute concentration.
- Osteoblasts** Bone-forming cells.
- Osteoclasts** Cells that break down or resorb bone.
- Osteomalacia** A disorder characterized by bone mineralization defects that may occur in adults because of inadequate vitamin D intake.
- Oxidation** An enzymatic reaction in which oxygen is added to, or hydrogen and its electrons are removed from, the reactant.
- Oxidative phosphorylation** The pathway in the mitochondria that makes ATP from ADP and P_i .
- Oxidoreductases** Enzymes that catalyze all reactions in which one compound is oxidized and another is reduced.
- Oxygenation reactions** Reactions that involve the introduction of or require one or more oxygen atoms.
- Parenchymal cells** The functional cells of an organ such as the liver.
- Pellagra** A condition that results from niacin deficiency.
- Peroxisomes** Cell organelles containing enzymes that perform oxidative catabolic reactions.
- Peroxyl radical** O_2^{2-} .
- Petechiae** Skin discolorations caused by ruptured small blood vessels.
- Phagocytosis** An endocytic process in which material is engulfed into a cell.
- Phospholipids** Lipids that belong to a class of complex lipids containing phosphate and one or more fatty-acid residues.
- Phosphorolysis** The process by which individual glucose units are sequentially released from glycogen.
- Phosphorylation** The metabolic process of adding a phosphate group to an organic molecule.
- Phytochemical** A biologically active, nonnutritive substance that is found in plants.
- Phytol tail** Refers to the structure of the side chains of vitamins E and K.
- Pinocytosis** Uptake of a substance into a cell through the formation of vesicles derived from the plasma membrane.
- Plasma membrane** The membrane encapsulating the cell.
- Polymer** A substance with a high molecular weight, made up of a chain of repeating units.
- Polysaccharides** Long chains of monosaccharide units that may number from several into the hundreds or thousands.
- Porphyrin** The nitrogen- and iron-containing nonprotein portion of hemoglobin.
- Postprandial** Occurring after a meal.
- Potentiometry** A method using electrodes that enables direct measurement of various anions and cations such as potassium, sodium, and chloride.
- Preprandial** Occurring before a meal.
- Probiotics** Products that contain specific strains of microorganisms in sufficient numbers to alter the microflora of the gastrointestinal tract, ideally to exert beneficial health effects.
- Prokaryotic cells** Primitive cells that do not contain a defined nucleus.
- Propagation** The ongoing generation of free radicals following the initiation stage of free radical formation.
- Prophylactic** A substance or regime that helps to prevent disease or illness.
- Prostaglandins** Biologically active compounds derived from arachidonic acid.
- Proteases** Enzymes that digest (break down) proteins.
- Protein kinases** A family of enzymes that transfers a phosphate group to another protein from ATP.
- Proteoglycans** Large molecules made up of proteins and glycosaminoglycans.
- Proteolytic** The breakdown of protein.
- Quenching** A process by which electronically excited molecules, such as singlet molecular oxygen, are inactivated.
- Receptors** Macromolecules (usually proteins) that bind a signal molecule with a high degree of specificity that triggers intracellular events.
- Recommended dietary allowance** The average daily dietary intake level of a nutrient that is thought to be sufficient to meet the nutrient requirements of about 97% of healthy individuals.
- Reflex** An involuntary response to a stimulus.
- Reperfusion** The resupply of an organ or tissue with oxygen, nutrients, or both.
- Replication** The synthesis of a daughter duplex DNA molecule identical to the parental duplex DNA.
- Resin** A compound that is usually solid or semisolid and usually exists as a polymer.
- Respiratory quotient (RQ)** The ratio of the volume of CO_2 expired to the volume of O_2 consumed.
- Rhodopsin** A vitamin A-containing protein found in the eye.
- Rickets** A condition in infants and children that results from vitamin D deficiency.
- Ryanodine receptor** A calcium channel in the sarcoplasmic reticulum of muscle that opens to permit the release of calcium.
- Sarcoplasmic reticulum** The smooth endoplasmic reticulum that is found in muscle cells and is the site of the calcium pump.
- Scintillation counter** An instrument used to measure concentrations of radioactive isotopes in a sample.
- Scurvy** A condition resulting from vitamin C deficiency.
- Seborrheic dermatitis** An inflammatory skin condition.
- Sense strand** The strand of DNA that serves as a template for mRNA.
- Short-chain fatty acids** Fatty acids typically containing two to four carbons.
- Sideroblastic anemia** An inherited disorder that affects red blood cell production and function.
- Singlet molecular oxygen** An electronically excited radical in which one of oxygen's electrons is excited to an orbital above the one it normally occupies.
- Sphingolipids** Phospholipids that contain the amino alcohol sphingosine, rather than glycerol.
- Splanchnic** Pertaining to the internal organs (viscera), especially the intestines.
- Standard reduction potential** The tendency of a molecule to donate or receive electrons.
- Steatorrhea** The presence of an excessive amount of fat in the feces.

- Stellate cells** Storage cells of the liver.
- Stereoisomers** A group of compounds that have the same structure but different configurations.
- Sterols** A subclass of lipids that contain a cyclopentanoperhydrophenanthrene ring system, a hydroxyl group, and a side chain.
- Substrate-level phosphorylation** The process of transferring a phosphate group from one organic molecule to another.
- Superoxide radical** An oxygen-centered free radical, O_2^- .
- Teratogenic** Causing birth defects in a fetus.
- Tetany** A condition resulting from inadequate blood calcium concentrations, characterized by prolonged muscle contraction.
- Thalassemia** A hereditary form of anemia associated with defective synthesis of hemoglobin.
- Thermogenesis** The production of heat within the body.
- Thermoregulation** A regulatory mechanism that keeps heat production and loss about equal.
- Thiobarbituric acid reactive substances** Compounds such as hexanal, pentanal, or pentane that react with thiobarbituric acid and suggest oxidative damage has occurred.
- Thromboxanes** Biologically active compounds derived from arachidonic acid.
- Tolerable upper intake level** The highest daily intake level that is likely to cause no risk of adverse health effects to most individuals in the general population.
- Tonic** Pertaining to or characterized by tension or contraction.
- Transcalcitania** Rapid intestinal calcium absorption stimulated by the active form of vitamin D.
- Transcription** The process by which the genetic information (base sequence) in a single strand of DNA is used to specify a complementary sequence of bases in an mRNA chain.
- Transducin** A G-protein, found in the eye, that responds to changes in opsin and is involved in the visual cycle.
- Transferases** Enzymes that catalyze reactions not involving oxidation and reduction, in which a functional group is transferred from one substrate to another.
- Transition state** Energy level at which reactant molecules have been activated and can undergo an exothermic reaction.
- Translation** The process by which genetic information in an mRNA molecule specifies the sequence of amino acids in the protein product.
- Translocation** Movement of a compound or agent across a cell membrane, such as the intestinal cell, and into the blood.
- Transport proteins** Proteins that transport nutrients in blood or into and out of cells or cell organelles.
- Tropical sprue** A disease common in tropical regions and characterized by weakness, weight loss, poor nutrient digestion and absorption, and steatorrhea.
- Ubiquinol** The alcohol form of ubiquinone, a fat-soluble molecule that functions in electron transport and ultimately ATP generation; also called *coenzyme Q₁₀* or *CoQ₁₀*.
- Ubiqutin** A protein that attaches to other proteins within cells or tissues to promote the degradation of the protein.
- Vascular system** The circulatory pathway that delivers blood to and from organs.
- VO₂ max** The maximal uptake of oxygen, as measured during a test with increasing work intensity.
- Ward's triangle** A region within the pelvis (hip).
- Xenobiotics** Foreign chemicals such as drugs, carcinogens, pesticides, food additives, pollutants, or other noxious compounds.
- Xerophthalmia** Dryness of the conjunctiva and keratinization of the epithelium of the eye following inflammation of the conjunctiva associated with vitamin A deficiency.
- Zwitterion** An amino acid with no amino or carboxyl groups in its side chain.
- Zymogen** An inactive form of an enzyme, also referred to as a *proenzyme*.

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